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Ion Flux Rates, Acid–Base Status, and Blood Gases in Rainbow Trout, *Salmo gairdneri*, Exposed to Toxic Zinc in Natural Soft Water

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Spry, D. J., and C. M. Wood. 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

Exposure to 0.8 mg Zn^{2+}/L in natural soft water for up to 72 h was toxic to rainbow trout, *Salmo gairdneri*, causing an acid–base disturbance and net branchial ion losses. Mean arterial pH fell from 7.78 to 7.58. Both Pa_{CO_2} and lactate rose, indicating a mixed respiratory and metabolic acidosis, despite maintenance of high Pa_{O_2}. Net branchial uptake of Na^+ and Cl^- became a net loss immediately following exposure to Zn^{2+}, and this continued during 60 h of exposure. Net K^+ loss was exacerbated, and net Ca^{2+} uptake was abolished. Unidirectional flux measurements with $^{22}Na^+$ and $^{36}Cl^-$ indicated an increased efflux immediately following zinc exposure. Both influx and efflux of Na^+ and Cl^- were stimulated after 48–60 h in Zn^{2+}. Both net branchial ammonia excretion and net branchial uptake of acidic equivalents from the water (=base loss) were greatly stimulated, the latter contributing to metabolic acidosis. Kidney function, as measured by urine flow rate and excretion of ammonia, acidic equivalents, Na^+, Cl^-, K^+, and Zn^{2+}, was relatively insensitive to the effects of zinc. The only renal component to be affected was Ca^{2+} excretion, which decreased during a single flux period, possibly in response to the reduced entry of Ca^{2+} at the gill. We conclude that toxic concentrations of zinc are capable of altering gill function so as to cause ionoregulatory and acid–base disturbances without disturbance of Pa_{O_2}.

L'exposition de truites arc-en-ciel, *Salmo gairdneri*, à 0,8 mg de Zn^{+2} par litre d'eau douce naturelle pendant 72 h a eu une incidence toxique, c.-à-d. une perturbation de l'équilibre acide–base et des pertes nettes d'ions branchiaux. Le pH artériel moyen est passé de 7,78 à 7,58 tandis que le Pa_{CO_2} et le lactate ont augmenté, ce qui indique une acidose respiratoire et métabolique hétérogène malgré le maintien d'un Pa_{CO_2} élevé. La captation nette de Na^+ et de Cl^- au niveau des branchies est devenue une perte nette immédiatement après l'exposition à du Zu^{+2} et pendant 60 h d'exposition. La perte nette de K^+ a été aggravée et la captation nette de Ca^{+2}, supprimée. Des quantifications du flux unidirectionnel de $^{22}Na^+$ et de $^{36}Cl^-$ ont révélé un écoulement accru immédiatement après l'exposition au zinc. L'entrée et la sortie de Na^+ et de Cl^- ont été stimulées après une exposition au Zn^{+2} allant de 48 à 60 h. L'excrétion nette d'ammoniaque branchial et la captation nette d'équivalents acides du milieu au niveau des branchies (= perte de base) ont été grandement stimulées; cette dernière a contribué à l'acidose métabolique. La fonction rénale, telle que mesurée par le taux d'évacuation de l'urine et l'excrétion d'ammoniaque, d'équivalents acides, de Na^+, de Cl^-, de K^+ et de Zn^{+2}, a été relativement insensible aux effets du zinc. L'excrétion du Ca^{+2} était la seule composante rénale touchée : elle a diminué au cours d'une seule période de flux, probablement en réaction à la réduction de l'entrée de Ca^{+2} au niveau des branchies. Les auteurs formulent la conclusion que des concentrations toxiques de zinc sont capables de modifier la fonction des branchies et de causer des perturbations de l'ionorégulation et de l'équilibre acide–base sans modifier le Pa_{O_2}.

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Concentrations of waterborne zinc that are rapidly lethal to trout severely disrupt gill tissue (Skidmore and Tovell 1972) and hence oxygen transfer by imposing a diffusion limitation for oxygen. The result is hypoxia (Skidmore 1970) and acidemia (Sellers et al. 1975; Spry and Wood 1984). The effects upon ion regulation are less clear. Skidmore (1970) found small but significant changes in plasma osmotic pressure in rainbow trout, *Salmo gairdneri*, exposed to 40 mg Zn^{2+}/L, but discounted these as being unimportant in the rapidly developing lethality. Spry and Wood (1984) reported no significant changes in major blood electrolytes in dying trout which showed hypoxemia and acidosis in 1.5 mg Zn^{2+}/L when compared with controls. This might have been obscured by the observed hemoconcentration. However, at a lower concentration (0.8 mg Zn^{2+}/L), where acute hypoxemic death did not occur, plasma Ca^{2+} levels decreased over a 3-d exposure but other plasma ions were unaffected. Lewis and Lewis (1971) noted a decrease in the serum osmolality of channel catfish (*Ictalurus punctatus*) exposed to lethal Zn^{2+} solutions (12–30 mg/L). When they added NaCl to the water to create an external

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osmotic pressure of 235 mosmol/L, mortality was delayed. This latter study suggests that regulation of Na^+ and/or Cl^- might indeed be affected, and possibly be of primary importance, under conditions where oxygen delivery was not clearly limiting.

Zinc might exert such an effect upon ion regulation by altering ATPase activities. Watson and Beamish (1981) showed in vitro inhibition of various ATPases in freshwater-adapted rainbow trout, although an earlier study (Watson and Beamish 1980) showed increased activity in vivo after 30 d in Zn^{2+}. This increased activity was suggested to be secondary to a Zn^{2+}-induced increase in gill permeability even though serum osmolality and electrolytes were unchanged. Zinc also inhibited chloride transport across the isolated opercular epithelium of seawater-adapted *Fundulus heteroclitus*, possibly as a consequence of its inhibitory effect in vitro upon Na^+,K^+-ATPase (Crespo and Karnaky 1983).

It is well known that zinc toxicity increases with decreasing hardness and alkalinity (Spear 1981). In the very soft water of Ontario Precambrian Shield lakes, zinc enrichment accompanying acidification may become a problem (Spry et al. 1981). The effects of zinc exposure on rainbow trout in *artificial* soft water have been reported earlier (Spry and Wood 1984). To compare these results with exposure in *natural* soft water of the Shield area, we completed a series of experiments at a field site which, in addition, examined the effects of a low level of Zn^{2+} (0.8 mg/L) upon ionoregulation. Our objectives were firstly, to measure acid–base, ionic, blood gas, and other blood variables in rainbow trout fitted with dorsal aortic cannulae and secondly, to separate branchial and renal net ammonia and acidic equivalent fluxes, as well as net and unidirectional ion fluxes, in trout with bladder catheters. We chose a zinc concentration close to the 96-h LC50 for comparison with the previous study (Spry and Wood 1984). Branchial and renal flux rates have not previously been measured in fish exposed to Zn^{2+}.

Materials and Methods

Rainbow trout underyearlings were procured from Skeleton Lake Hatchery or Milford Bay Trout Farm in late summer. Both hatcheries had soft water (Ca^{2+} = 0.2 mequiv/L). Fish were moved by truck to Harkness Laboratory in Algonquin Park (latitude 45°42′; longitude 78°23′) where they were kept in tanks supplied with flowing Lake Opeongo water at 17–22°C. A commercial pelleted diet (Martin Feed Mills, Elmira, Ont.) was fed daily. This was the same diet as used in our previous study (Spry and Wood 1984). Trout were acclimated to 15°C for at least 4 d in a flow-through system chilled with Min-o-cool units (Frigid Units). Food was withheld 2 d prior to any surgery and throughout the experimental period.

Blood Measurements

To assess the effects of Zn^{2+} exposure on arterial blood composition, we cannulated the dorsal aorta (Smith and Bell 1964) of rainbow trout (141–267 g) under MS222 anesthesia and placed each fish in one of eight individual compartments of a black acrylic box, allowing them to recover for 36–48 h. Cannulae were periodically flushed with heparinized (ammonium heparin, 100 IU/mL) Cortland saline (Wolf 1963). Water was circulated to the fish from a common head tank at 200 ± 16 mL·min^{-1}·fish^{-1}, collected in a sump tank, and then cooled, aerated, and returned to the head tank. Total volume of the system was 70–115 L, and there were no metal parts in contact with the water. A continuous slow input to the recirculating system of fresh lake water, or lake water plus toxicant, provided a 90% replacement time of 12–14 h (Sprague 1973). Water quality characteristics representative of the "best" and "worst" cases (i.e. start of the experiment, and in one trial where the system was closed for 24 h) are given in Table 1; typical experimental values lay between these extremes.

TABLE 1. Some water quality measurements under control conditions, means ± SE (*n*).

Variable	Lake Opeongo water prior to start of experiment	Lake Opeongo water from test battery after 24 h with fish in place, without replacement
Na^+ (mequiv/L)[a]	0.067±0.003 (5)	0.089±0.011 (6)
Cl^- (mequiv/L)[b]	0.028±0.003 (5)	0.075±0.029 (6)
K^+ (mequiv/L)[a]	0.020±0.001 (5)	0.056±0.018 (6)
Ca^{2+} (mequiv/L)[a]	0.170±0.001 (5)	0.170±0.012 (6)
NH_4^+ (mequiv/L)[c]	0.040±0.004 (4)	0.190±0.050 (4)
NO_2^- (mequiv/L)[d]	—[e]	0.001±0.005 (4)
Al (μg/L total)[f]	6.46	10.66
Zn (μg/L total)	<50	<50
Conductivity (μS/cm)	46.90±0.48 (8)	75.25±6.19 (8)
Temperature (°C)	15.5±0.3 (12)	15.5±0.3 (12)
pH[g]	6.78±0.27 (6)	7.32±0.05 (8)
Alkalinity (μequiv/L)[h]	95	282

[a]Measured by flame photometry.
[b]Measured by coulometric titration.
[c]Method of Verdouw et al. (1978).
[d]Standard methods (APHA et al. 1975).
[e]Not measured.
[f]Lumogallion fluorescence method of Playle et al. (1982).
[g]Statistics performed on [H^+] and converted to pH.
[h]Single measurement, inflection point titration.

Blood variables were measured daily for 4 d. After the first sample (control), reagent-grade $ZnSO_4 \cdot 7H_2O$ was added to the head tank to give 0.8 mg/L. The blood sampling protocol consisted of drawing 0.6 mL of arterial blood into gas-tight Hamilton syringes for determinations of arterial pH (pHa), total CO_2 (Ca_{CO_2}), oxygen partial pressure (Pa_{O_2}), hematocrit (hct), and concentrations of hemoglobin ([Hb]) and lactate. An additional 0.3 mL of sample was centrifuged for 5 min at 8000 × *g* and plasma removed for analysis of Na^+, Cl^-, K^+, Ca^{2+}, and total protein (C_{Pr}). The pellet of red blood cells (rbc) was resuspended in heparinized Cortland saline and the rbc returned to the fish to reduce loss due to sampling. Hemolysis occurred rarely, and these samples were discarded. Here, saline alone was infused to maintain extracellular fluid volume. On average, the total whole blood removed at each sample time was 0.6 mL, of which the rbc from 0.3 mL were returned. Data from fish whose hct fell below 5% were discarded, since anemia itself can provoke an acidosis (Wood et al. 1982). In fact, only 2 of 12 final hct values were <9%, and the mean was 13.5 ± 1.7% (12).

Branchial and Renal Flux Rates

To separately measure branchial and renal flux rates of ions and "acidic equivalents" under control conditions and subsequent exposure to 0.8 mg Zn^{2+}/L, we implanted only bladder catheters (Wood and Randall 1973b), using MS222 anesthesia, in a second group of fish. Each fish was then placed in a flux box (McDonald 1983a), consisting of a small inner box of clear

acrylic which confined the fish and a larger outer box of black acrylic which held 6–8 L of water. Boxes were placed on a wet table flooded with cool well water to maintain 15–17°C. An airlift pump at the rear of the inner box, together with perimeter aeration within each outer box, ensured circulation and maintained $Pw_{O_2} > 120$ Torr. Urine was collected in 50-mL flasks outside the boxes. Thus, changes in the composition of the water during the flux period reflected net branchial fluxes.

Following 3–4 d of recovery, three control fluxes were monitored, followed by five treatment (0.8 mg Zn^{2+}/L) fluxes of 12 h each. Variation within the three control periods was minimal, and composite control means are reported. Boxes were flushed with fresh solution (lake water or toxicant) at 12-h intervals, providing 70–80% replacement, based upon ammonia dilution. Since this procedure took ~2 h, the branchial fluxes were actually measured over 10 h, whereas the renal collections were throughout the entire 12-h period. Samples for water titratable alkalinity or urine titratable acidity minus bicarbonate ([TA − HCO_3^-]) were stored at 4°C and analyzed within 24 h. The remainder were frozen (−20°C) for later analysis.

Unidirectional branchial fluxes of Na^+ and Cl^- were partitioned after Kirschner (1970) during the first control flux period, flux period 1 (step change to Zn^{2+}), and flux period 5 (2 d of Zn^{2+} exposure). One hundred and eighty five kilobequerels (5 μCi) of $^{22}Na^+$ and 92.5 kBq (2.5 μCi) of $^{36}Cl^-$ (New England Nuclear) were added to each flux box and mixed thoroughly, and water samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, and 5 h.

Analytical Methods

Whole blood and plasma Ca_{CO_2} (Cameron 1971), pHa, and Pa_{O_2} were measured on a Radiometer PHM 71 acid–base analyzer fitted with gas modules. The pH and O_2 electrodes were water-cooled to the experimental temperature, while the CO_2 electrode in the Cameron chamber was maintained at 37°C. The pH electrode was calibrated frequently with precision buffers. The O_2 electrode was calibrated with water-saturated nitrogen or air and the Cameron chamber by known bicarbonate addition. L-(+)-lactate was determined enzymatically (LDH/NADH, Sigma Technical Bulletin 726UV/826UV) after deproteination of whole blood in 8% $HClO_3$. Hemoglobin was measured as cyanmethemoglobin (Sigma Technical Bulletin 525) using Sigma or Hycel reagents. Hematocrit was measured by centrifuging blood in heparinized microhematocrit tubes at 5000 × *g* for 5 min. The mean cell hemoglobin concentration (MCHC) was calculated as [Hb]/hct × 100 (Dacie and Lewis 1975). Plasma total protein determination was by refractometry (American Optical). Plasma Cl^- was titrated on a chloridometer (Radiometer CMT-10). After suitable dilution and swamping to eliminate interference effects, plasma Na^+ and K^+ were measured on an EEL mk 2 flame photometer. Plasma Ca^{2+} was measured by colorimetry (Sigma Technical Bulletin 585).

Major ions (Na^+, K^+, Ca^{2+}, Cl^-) in water and urine were measured as for plasma except for water Cl^-, which at very low levels was assayed with a Buchler–Cotlove chloridometer. The acid reagent had 0.2 mmol NaCl/L added to provide a linear response. Ca^{2+} and Zn^{2+} in the water and urine were determined by atomic absorption spectrophotometry (Varian AA1275, or initially for Zn^{2+}, a Jarrel-Ash 800). Total water and urine ammonia levels were determined using a micromodification of the salicylate–hypochlorite method of Verdouw et al. (1978). For titratable alkalinity, 10 mL of water was gently aerated and titrated with 0.02 mol HCl/L from a Gilmont burette to <pH 4, and the volume of titrant required to titrate to pH 4 was interpolated (DeRenzis and Maetz 1973; McDonald and Wood 1981). Aeration throughout titration ensured CO_2 removal. Titration of urine was by the single step determination of titratable acidity minus bicarbonate ([TA − HCO_3^-]) (Hills 1973) in which sufficient 0.02 mol HCl/L was added to 500 μL of urine to drive the pH to <4. This was then aerated for CO_2 removal and titrated back through the pHa (mean day 0 value, acid–base experiment) with freshly standardized 0.02 mol NaOH/L. The volume of titrant at pHa was interpolated and the volume of the acid added was subtracted.

For unidirectional flux measurements, water samples were counted as follows. $^{36}Cl^-$ is a pure beta emitter, while $^{22}Na^+$ is a mixed beta and gamma emitter. Dual labelled water samples were prepared in duplicate, with $^{22}Na^+$ alone measured in a well-type counter (Nuclear-Chicago model 1085) and $^{22}Na^+$ plus $^{36}Cl^-$ by scintillation counting (Beckman LS 250). After correction for difference in efficiency of $^{22}Na^+$ counting by the two machines, the $^{36}Cl^-$ counts were obtained by subtraction.

Calculations

The partial pressure of CO_2 in arterial blood (Pa_{CO_2}), the HCO_3^-, and the total blood metabolic acid load (ΔH^+_m) were calculated using standard acid–base equations as described by Spry and Wood (1984). Values for α_{CO_2} (CO_2 solubility in plasma), pK_1' (apparent first dissociation constant of carbonic acid), and β (nonbicarbonate buffering capacity) at a particular blood [Hb] were taken from Severinghaus (1965), Albers (1970), and Wood et al. (1982), respectively.

Net branchial ion fluxes observed from changes in waterborne concentrations were calculated as follows:

$$(1)\quad J_{net} = \frac{[X]_i V_i - [X]_f V_f}{t \cdot W}$$

where $[X]$ is the ion concentration (microequivalents per litre), V is the box volume which decreases during the period due to sampling, subscripts i and f are initial and final, respectively, t is the duration of the flux period (hours), and W is the fish weight (kilograms). Thus, net losses by the animal have a negative sign and net gains a positive sign. For titratable alkalinity, $V_t N / V_s$ is substituted for $[X]$, where V_t is the titrant volume (millilitres), N is the acid normality (microequivalents per litre), and V_s is the sample volume (millilitres). By reversing the i and f terms, the net titratable acidity flux was calculated from the titratable alkalinities. The net acidic equivalent flux is the sum of titratable acidity flux and ammonia flux, signs considered (cf. McDonald and Wood 1981).

For unidirectional branchial fluxes, J_{in} was calculated from the natural logarithm function given by Kirschner (1970), since no measurable backflux of isotope occurred:

$$(2)\quad J_{in} = \frac{Q_{out}}{t \cdot W} \cdot (\ln Q^*_{out(0)} - \ln Q^*_{out(t)})$$

where Q_{out} is total amount of the desired ion in the medium and Q^*_{out} is the total amount of radioactivity (cpm) at time 0 and t, respectively. J_{out} (negative by convention) was calculated as $J_{net} - J_{in}$. Renal ion losses were the product of concentration and urine flow rate (UFR) whereas the renal net acidic equivalent flux is given by

$$(3)\quad J_{out} = ([TA - HCO_3^-] + [NH_4^+]) \cdot UFR,$$

total ammonia being considered equivalent to $[NH_4^+]$, as free NH_3 was negligible at urine pH.

All values are reported as mean ± 1 standard error. To assess significant differences, we used paired Student's *t*-test where possible, since each fish served as its own control. Where missing values made this impossible, unpaired *t*-tests were used, with a resultant loss of power (Steel and Torrie 1960).

Results

Exposure to 0.8 mg Zn^{2+}/L in natural soft water resulted in significant blood acidosis, a decrease in plasma Ca^{2+}, increase in plasma K^+, and altered branchial ion and acidic equivalent fluxes. In contrast, there was little effect upon renal fluxes. The exposure was toxic, and overall mortality in 31 fish was 53% over 3 d. For blood data, values for all fish, and for those that survived the 3-d exposure, are shown separately in Fig. 1–3. Differences between the two data sets were not significant. The response of the survivors was therefore representative of the experimental population as a whole. For branchial and renal fluxes, only data from surviving animals have been plotted in Fig. 4–6 and Table 2 for the sake of clarity. Flux rates in dying fish showed similar trends, but generally increased and/or became highly erratic prior to death. Due to time constraints in the field situation, parallel controls were not run. However, under similar conditions in artificial softwater, the protocol caused neither mortality nor acid–base nor ionic disturbance (Spry and Wood 1984).

Examination of the acid–base status of fish that survived to the end of the experiment (Fig. 1a–1c) revealed a gradual and progressive decline in pHa which became significant on the final day. It was primarily respiratory in nature as shown by the rise in Pa_{CO_2}, with no significant change in plasma bicarbonate. Analysis of means plotted on a pH–bicarbonate diagram (Davenport 1974, not shown) indicated that the acidosis was mixed respiratory and metabolic, with the respiratory component becoming significant on the last day (Fig. 1c). The small but significant rise in blood lactate (Fig. 1e) was consistent with a metabolic component although the blood metabolic acid load was not significantly increased (Fig. 1f). The Pa_{O_2} remained uniformly high (Fig. 1d), a surprising finding in light of the increase in both Pa_{CO_2} and lactate. While ventilation was not directly assessed, there was no noticeable hyperventilation that might be associated with hypoxemia.

Additional blood parameters (Fig. 2) all showed significant declines with time. Similar declines were noted for hct, Hb, and C_{Pr} under control conditions (Spry and Wood 1984) which were attributable to the sampling protocol. This indicates that no significant hemoconcentration occurred in the present study, in contrast with that seen in more acutely lethal exposures at higher zinc levels (Spry and Wood 1984). However, MCHC did fall significantly (Fig. 2c), suggesting either that rbc swelling accompanied the developing acidosis or that a mobilization of Hb-poor rbc (e.g. reticulocytes) occurred.

Of the major plasma ions, Na^+ and Cl^- showed some fluctuation (Fig. 3a, 3b) but were essentially unchanged. However, K^+ rose significantly on the last day (Fig. 3c), while Ca^{2+} fell significantly after only 1 d (Fig. 3d), a trend that became even more pronounced over the following days.

Under control conditions, branchial ammonia excretion was ~200–250 $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ (Fig. 4), or about 20-fold higher than renal ammonia efflux (Table 2). This was approximately balanced by the titratable acidity "uptake" (Fig. 4), such that

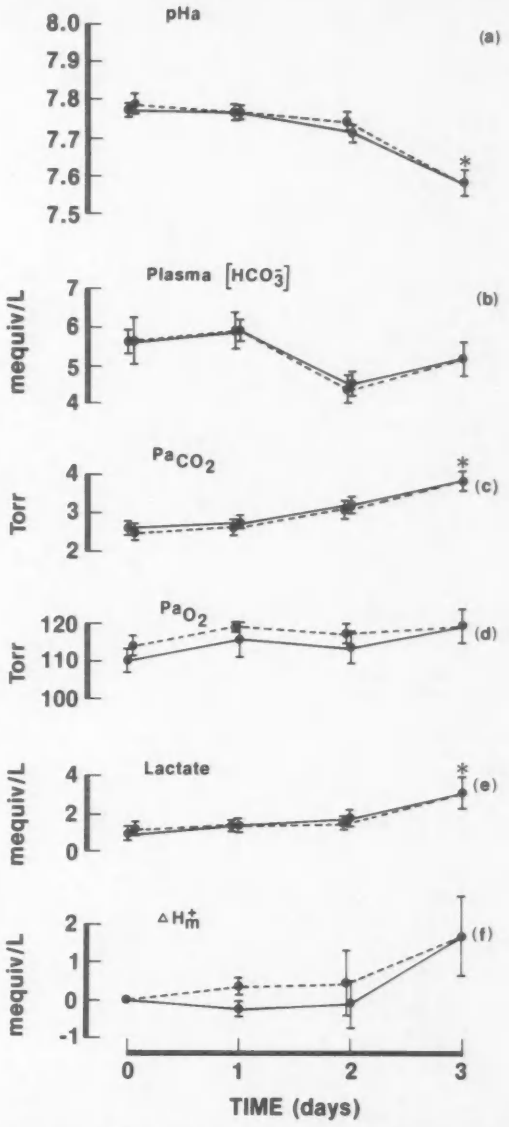

FIG. 1. Arterial blood measurements (a) pHa, (b) plasma $[HCO_3^-]$, (c) Pa_{CO_2}, (d) Pa_{O_2}, (e) lactate, and (f) metabolic acid load in rainbow trout under control conditions (day 0) and subsequently exposed to 0.8 mg Zn^{2+}/L. Means ± SE are shown for survivors to day 3 (n = 11, solid line) or all fish (n = 30, 24, 22, and 11 for day 0 to day 3, respectively). Asterisks denote means significantly different from control values ($p < 0.05$).

the net branchial acidic equivalent flux was either close to or slightly above zero (Fig. 4). Upon Zn^{2+} exposure, both components slowly increased over the following 3 d. However, the titratable acidity "uptake" increased to a greater extent than the ammonia efflux, so that an increasingly positive net acidic equivalent flux occurred, which reached approximately +200 $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ by the final period.

The branchial net fluxes for Na^+, Cl^-, and Ca^{2+} (Fig. 5a, 5b, 5d) were positive under control conditions, representing a net

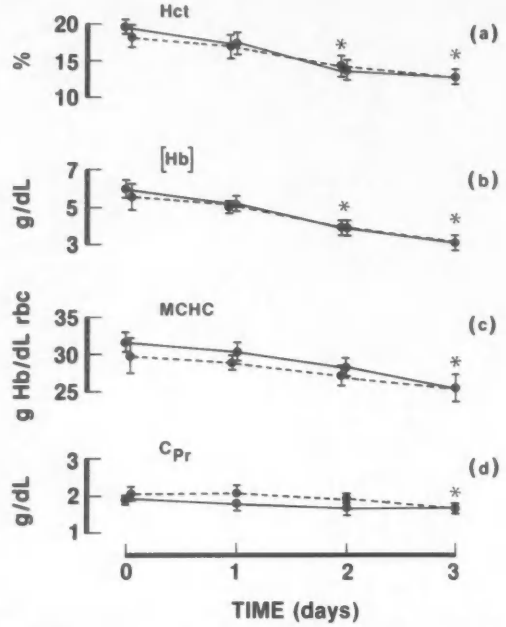

FIG. 2. Arterial blood measurements (a) hematocrit, (b) hemoglobin, (c) mean cell hemoglobin concentration, and (d) plasma total protein in rainbow trout under control conditions (day 0) and subsequently exposed to 0.8 mg Zn^{2+}/L in natural soft water. Means ± SE are shown for survivors to day 3 (n = 12, solid line) or all fish (n = 29, 24, 22, and 12 for day 0 to day 3, respectively). Statistics as in Fig. 1.

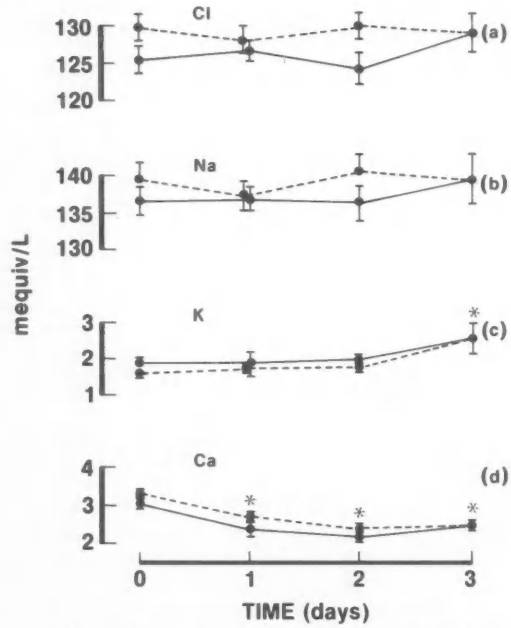

FIG. 3. Plasma ions (a) Cl^-, (b) Na^+, (c) K^+, and (d) Ca^{2+}. Legend as for Fig. 1 except for K^+ (survivors, solid line, n = 14; all fish, broken line, n = 30, 24, 22, and 14) and Ca^{2+} (survivors, n = 3; all fish, n = 13, 9, 6, and 3, on day 0 to day 3, respectively). Statistics as in Fig. 1.

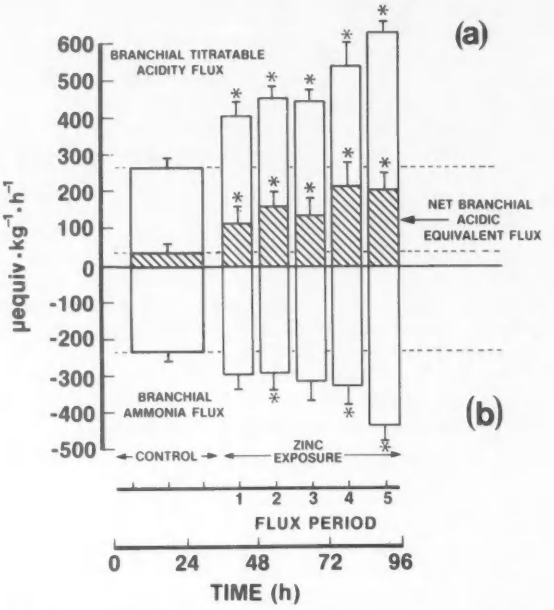

FIG. 4. Branchial net acidic equivalent flux components (a) titratable acidity flux, (b) ammonia flux, and the sum of (a) plus (b), the net acidic equivalent flux, in rainbow trout under control conditions (composite means) and during five subsequent fluxes in 0.8 mg Zn^{2+}/L, means ± SE, n = 13. Asterisks denote means significantly different ($p < 0.05$) from the composite control means (broken lines).

uptake from the water of approximately 20–40 μequiv·kg^{-1}·h^{-1}. K^+ (Fig. 5c) was an exception, exhibiting small but uniform losses. Exposure to Zn^{2+} immediately induced net losses in Na^+ and Cl^- and abolished Ca^{2+} uptake, but K^+ flux was not immediately affected. During continued Zn^{2+} exposure, the losses of Na^+ and Cl^- showed some sign of recovery after 36 h but then increased again in the last two flux periods. Overall Cl^- losses were greater than those of Na^+. K^+ losses only became significantly larger than the control mean during the last two flux periods. Net Ca^{2+} flux fluctuated near zero throughout the Zn^{2+} exposure. Two fish that were followed for an additional flux period (not shown) showed the same response for Na^+, Cl^-, and K^+ but interestingly, a positive net Ca^{2+} uptake indicating some potential for recovery.

Unidirectional flux measurements to partition Na^+ and Cl^- fluxes into efflux and influx components were performed in the first control flux period, flux period 1 (abrupt change to Zn^{2+}), and flux period 5 (2 d exposure) although not in all fish (Fig. 6). The abrupt change to Zn^{2+} increased Na^+ efflux alone, while continued exposure resulted in increases of both influx and efflux components. Effects upon Cl^- fluxes were similar, but with both components increasing immediately following Zn^{2+} exposure. In neither case were net fluxes significantly altered, in contrast with the pooled results above (Fig. 5a, 5b) although the overall trends were similar. This may have been due to the higher variability, smaller sample size, or the fact that the fluxes were only measured over 6 h instead of 12 h.

Under control conditions, renal losses of Na^+, Cl^-, and net acidic equivalents approximately balanced the net branchial uptake rates of these ions, while renal Ca^{2+} losses were only about 35% of the uptake rate at the gills (Table 2 vs. Fig. 4 and

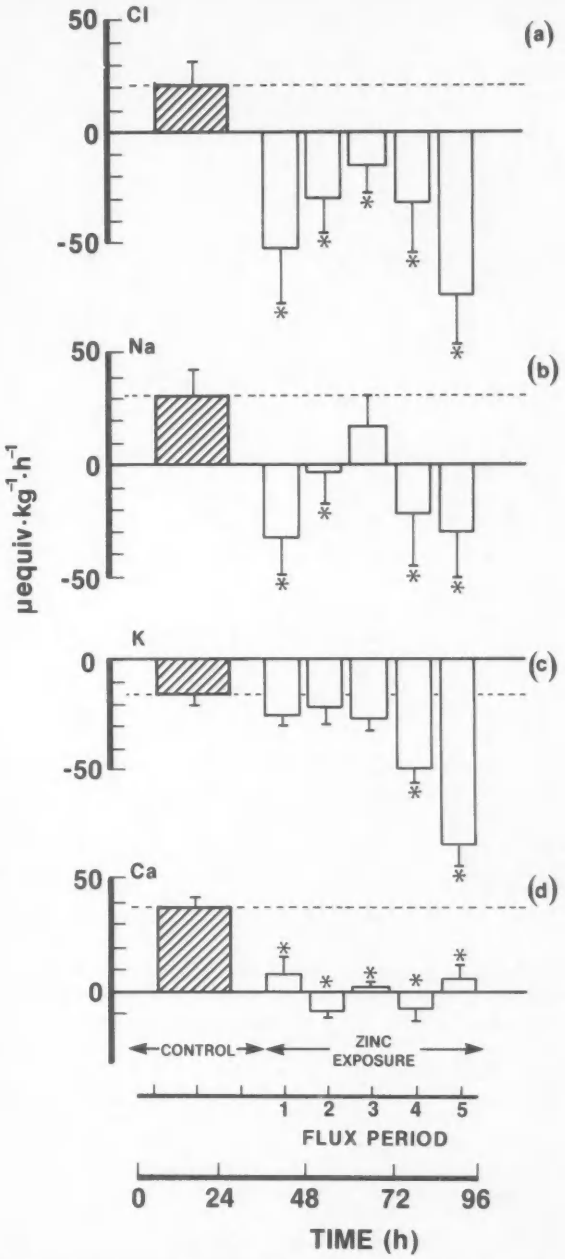

FIG. 5. Net branchial flux rates for (a) Cl^-, (b) Na^+, (c) K^+, and (d) Ca^{2+} in rainbow trout under control conditions (composite means) and subsequently exposed to 0.8 mg Zn^{2+}/L, means ± SE, $n = 13$ except for Ca^{2+} fluxes 3 and 5 where $n = 12$. Asterisks denote means significantly different ($p < 0.05$) from the control composite mean (broken lines).

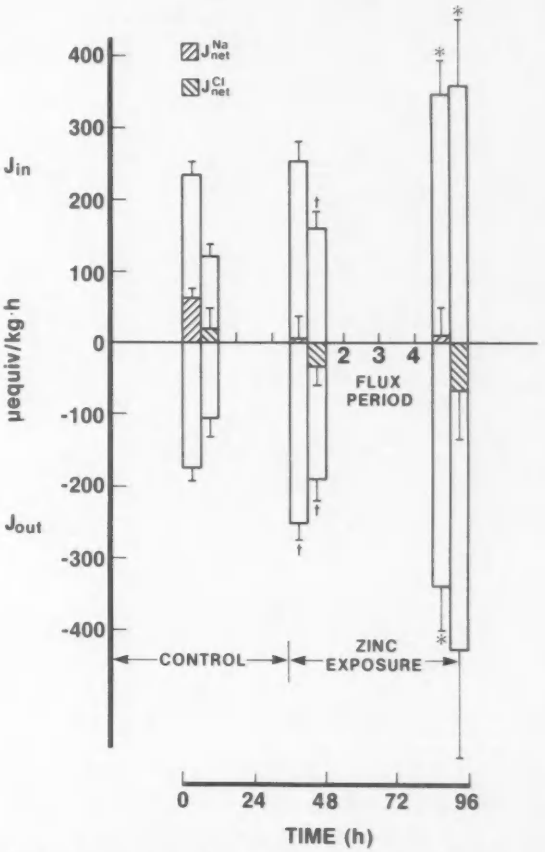

FIG. 6. Unidirectional (J_{in}, J_{out}) and net (J_{net}) branchial flux rates for Na^+ and Cl^- in rainbow trout under control conditions and subsequently exposed to 0.8 mg Zn^{2+}/L, means ± SE. For Na^+, $n = 13$, 13, and 7 for control flux 1, flux period 1, and flux period 5, respectively; for Cl^-, $n = 9$, 9, and 3. Significant differences ($p < 0.05$) from the control fluxes are denoted by daggers for paired t-test and asterisks for unpaired t-test.

5). Renal K^+ losses were only about 10% of renal Na^+ and Cl^- excretion rates, while renal Zn^{2+} excretion was about three orders of magnitude lower (Table 2). All the renal flux rates were relatively insensitive to Zn^{2+} exposure (Table 2). The only significant change was a decrease in Ca^{2+} efflux during flux period 5, possibly in response to decreasing entry of Ca^{2+} at the gill (Fig. 5d) and declining plasma Ca^{2+} levels (Fig. 3d). Notably there was no detectable change in the very low excretion of Zn^{2+} through the kidney, even over 60 h of exposure.

Discussion

The generally accepted consequence of exposure to acutely toxic waterborne Zn^{2+} (e.g. 1.5–40 mg/L) is an irreversible interruption of oxygen transfer across the gills caused by tissue damage. The resultant severe hypoxemia (Skidmore and Tovell 1972) with concurrent high lactate accumulation is fatal (Burton et al. 1972; Hodson 1976; Spry and Wood 1984). In the present study at 0.8 mg Zn^{2+}/L, significant mortality still occurred, although the Pa_{O_2} was unaffected (Fig. 1d) and the rise in blood lactate was rather small (Fig. 1e). This implies the presence of other toxic mechanisms which may be masked by the effects of higher concentrations of waterborne Zn^{2+}.

There are several possible explanations for the eventual small rise in blood lactate in the face of unchanged Pa_{O_2}. These

TABLE 2. Urine flow rates, and renal flux parameters in rainbow trout under control conditions and subsequently exposed to 0.8 mg Zn^{2+}/L in natural soft water. Values are means + SE(n). Units are $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ except where noted. *Significantly different from composite control mean ($p < 0.05$).

Variable	Composite control	Flux period 1	2	3	4	5
UFR ($mL \cdot kg^{-1} \cdot h^{-1}$)	6.59	5.77	6.06	6.77	5.40	7.70
	1.24	1.33	1.70	1.38	0.96	2.79
	9	9	9	9	9	9
[TA − HCO_3^-]	−1.76	−3.31	−2.92	−4.42	−1.08	2.10
	1.29	2.30	3.45	2.01	1.46	6.47
	7	7	6	7	4	4
Ammonia	7.82	5.60	7.12	6.69	4.75	10.57
	1.84	1.27	1.94	2.14	1.61	3.79
	7	7	6	7	4	4
Net acidic equivalents	6.50	2.28	4.20	2.27	3.67	12.66
	2.49	2.32	3.10	1.15	2.01	7.68
	7	7	6	7	4	4
Sodium	43.36	34.88	31.08	43.82	35.88	35.45
	11.60	11.71	8.72	11.75	9.48	9.42
	9	9	9	9	9	9
Chloride	39.33	27.69	26.11	30.24	21.72	24.22
	12.02	10.73	8.76	9.80	6.16	8.19
	9	9	8	9	9	9
Potasssium	4.34	3.40	3.95	4.18	4.20	5.92
	0.88	0.93	1.14	0.79	1.14	1.76
	9	9	8	9	9	9
Calcium	11.66	8.17	11.00	8.14	4.31*	10.34
	3.08	3.08	4.56	2.80	1.19	6.01
	9	9	8	9	9	9
Zinc ($nequiv \cdot kg^{-1} \cdot h^{-1}$)	16.15	19.92	21.01	18.37	11.65	15.83
	5.29	8.99	9.08	8.53	4.25	4.95
	9	7	8	7	7	7

include a diffusive limitation at the gill, decreased O_2 delivery to the tissues through a decreased arterial O_2 content or cardiac output, and finally, decreased utilization by the tissues. The latter is supported by evidence in the literature (Hiltibran 1971; Zaba and Harris 1978), although the experimental conditions are not necessarily comparable. The high Pa_{O_2} argues against a diffusive limitation, especially when compared with the dramatic fall in Pa_{O_2} reported in the other studies where Zn^{2+} exposure was rapidly lethal (Skidmore 1970; Sellers et al. 1975; Spry and Wood 1984) and structural gill damage clearly occurred (Skidmore and Tovell 1972). However, the constancy of Pa_{O_2} is not conclusive evidence against diffusive limitation, because blood O_2 capacity and/or flow rate could be simultaneously reduced to such an extent that a high Pa_{O_2} occurred despite a diffusive limitation. Indeed, blood O_2 capacity was undoubtedly reduced both by sampling-induced reduction in [Hb] and the progressively developing acidosis (i.e. Root effect) in our experiments. Definitive separation of these possible explanations will require simultaneous measurements of O_2 uptake, cardiac output, inspired and expired water P_{O_2}, and both arterial and venous blood O_2 contents and P_{O_2} levels.

The rise in Pa_{CO_2} (Fig. 1c) is unlikely to be due to simple diffusion limitations across the gill, since CO_2 is about 30-fold more soluble in water than is oxygen (Dejours 1975), and the Pa_{O_2} was unaffected. Branchial and/or erythrocytic carbonic anhydrase is thought to play a critical role in the excretion of CO_2 (Maetz 1971; Perry et al. 1981) and is known to be inhibited in vitro by Zn^{2+} (Christensen and Tucker 1976). Such inhibition by Zn^{2+} as a cause of the apparent decreased excretion of CO_2 in Zn^{2+}-exposed trout needs to be examined. Neither the acid–base disturbance nor the lactate accumulation was the cause of mortality, since severely exercised trout routinely experience much more severe acidosis and blood lactate elevation from which they usually recover (Wood et al. 1983).

The trout in this study were raised in natural soft water of the Ontario Precambrian Shield area and were exposed to 0.8 mg Zn^{2+}/L in this medium. In our previous study (Spry and Wood 1984), hardwater-reared trout were acclimated to artificial soft water and then exposed to the same level of Zn^{2+}. There was considerable similarity of the test conditions in terms of methodology, major water electrolytes, temperature, and pH. Although fish were of different stocks, both were thoroughly domesticated hatchery trout, fed identical diets. The two studies were similar in showing negligible effects on Pa_{O_2} and plasma Na^+ and Cl^-, significant increases in Pa_{CO_2} and decreases in plasma Ca^{2+}, and similar overall mortality. There were, however, some subtle but important differences. The fish in artificial

soft water developed an alkalosis rather than an acidosis, and increases in blood lactate and K^+ did not occur. These differences may have a genetic basis and/or may involve some property of natural soft water such as its complement of trace elements, other metals, or organic components. Whatever the explanation, these findings emphasize the importance of examining fish in their natural environment in studies of this nature. Hōbe et al. (1984) reached similar conclusions with respect to the effects of environmental acid stress in natural versus artificial soft water.

In addition to CO_2 excretion and acid–base status, branchial ion flux rates (Fig. 5) were also affected by exposure to Zn^{2+}, Cl^- apparently more so than Na^+. The unidirectional flux measurements (Fig. 6) indicated that initially, only the Cl^- influx component was stimulated, while the efflux component for both Na^+ and Cl^- increased. Longer exposure significantly elevated both Na^+ and Cl^- influx. The pooled net flux data showed that net losses of both ions clearly occurred at the gills (Fig. 5a, 5b). These losses were probably due to increased permeability of the gills to ions, possibly by opening paracellular channels. This potential for ionic losses exists for other waterborne toxicants such as copper (Sellers et al. 1975; Laurén and McDonald 1985), mercury (Lock et al. 1981), and environmental acid (Leivestad and Muniz 1976; McDonald 1983b).

After continued exposure, influx of both Na^+ and Cl^- as well as Na^+ efflux were stimulated. Watson and Beamish (1981) found Zn^{2+} to be generally inhibitory to a variety of ATPases in vitro, but a 30-d in vivo exposure had stimulatory effects (Watson and Beamish 1980). Whether the in vitro response was purely pharmacological while the in vivo response was part of a homeostatic mechanism is not known. The K^+ fluxes in the present study were not affected until near the end of the Zn^{2+} exposure when plasma K^+ levels were elevated (Fig. 3c) and mortality was high. It may thus represent general release from the intracellular fluids in response to acidosis (e.g. Ladé and Brown 1963) or specific release from the intracellular space of the branchial tissue due to damage of the apical (water facing) membrane. Histological condition of the gills was not examined.

The abrupt abolition of net Ca^{2+} uptake by Zn^{2+} exposure (Fig. 5d) and associated fall in plasma calcium levels (Fig. 3d) is particularly interesting. Since unidirectional fluxes were not measured, we can only speculate as to whether this resulted solely from reduced influx, increased efflux, or some combination of the two. If the former is true, then Zn^{2+} might be displacing Ca^{2+} as a substrate for the Ca-ATPase which has been isolated from the gill (Ma et al. 1974; Fenwick 1976). Such "accidental active uptake" was suggested for uptake into a freshwater amphipod (Wright 1980) for both Zn^{2+} and Cd^{2+}. The apparent recovery of Ca^{2+} uptake by two fish also raises important questions as to the cause of inhibition, and the potential of the gill to recover its transport function or reduce its permeability.

Table 3 summarizes the cumulative branchial and renal fluxes (relative to the control condition) over the 60 h of Zn^{2+} exposure. Clearly, the gills were the major sites of ion loss and net acidic equivalent uptake. Compensation by the kidney, in the form of reduced ion losses and elevated acid excretion, was minimal. The tabulation shows a significant movement of net charge (+4887 μequiv/kg) unaccounted for, almost entirely at the gills, requiring entry of an unmeasured anion or loss of an unmeasured cation to maintain electroneutrality. Hōbe et al. (1984) observed a very similar discrepancy in white suckers (*Catostomus commersoni*) exposed to acid stress in the same natural soft water although they used an opposite sign convention to express it.

TABLE 3. Total fluxes of ions and acidic equivalents, relative to control levels, in rainbow trout exposed to 0.8 mg Zn^{2+}/L in natural soft water for 60 h. All units are in μequiv/kg, corrected for control rates. For each ion species, sign represents gain (+) or loss (−) from the animal. For the net charge, sign represents gain or loss of positive charge.

	Branchial	Renal	Total
Na^+	−2789	+429	−2360
Cl^-	−3751	+800	−2951
Ca^{2+}	−2314	+196	−2118
K^+	−1502	0	−1502
H^+ [a]	+7831	+85	+7916
Net charge[b]	+4977	−90	+4887

[a]Net acidic equivalents.
[b]Net charge = $Na^+ + K^+ + Ca^{2+} + H^+ - Cl^-$.

The role of the gills in both acid–base and ion regulation is intimately linked via the $Na^+/H^+(NH_4^+)$, Cl^-/HCO_3^- exchanges (Maetz 1971; Maetz et al. 1976; Girard and Payan 1980; Wood et al. 1984). The stimulation of net acidic equivalent uptake (base excretion) during Zn^{2+} exposure (Fig. 4) was the opposite of expected, since the fish were acidotic and net excretion of acidic equivalents both at the gill (McDonald et al. 1983) and kidney (McDonald and Wood 1981) normally occurs in the face of an acid load. We suggest that Zn^{2+} interfered with normal exchanges at the gill. Possible effects include stimulation of base excretion (increased Cl^- influx relative to Na^+ influx, as was seen upon initial Zn^{2+} exposure, Fig. 6), inhibition of acid excretion (decreased Na^+ uptake relative to Cl^- uptake), and elevated passive proton entry, which would be favoured by the pH gradient between soft water (pH ~ 6.7–7.3) and blood (pH ~ 7.8). All these would contribute to the metabolic component of the observed blood acid–base disturbance.

Although ammonia is a base, its loss from the fish occurs either as NH_3, in which case it does not affect the acid–base status of the fish, or as NH_4^+, which carries out a proton, and is therefore acidic equivalent excretion. Thus, although ammonia excretion increased due to Zn^{2+} exposure (Fig. 4), its contribution to acid excretion (currently under some debate, cf. Cameron and Heisler 1983; Wright and Wood 1985) was not sufficient to counteract the net base loss.

The lack of response by the kidney (Table 2) (with the exception of decreased Ca^{2+} excretion during one period) in the face of increased branchial ion losses indicated net whole body ion depletion (Table 3). Exchangeable NaCl in freshwater trout is about 48 mequiv/kg (Wood and Randall 1973a; McDonald 1983a). The observed Na^+ and Cl^- losses were thus about 5% of the exchangeable pool over 60 h. From the constancy of the plasma ions (Fig. 3a, 3b) we suggest an isosmotic loss, or replenishment from the intracellular compartment. However, such a rate of loss clearly could not be sustained. The decreased losses of Na^+ and Cl^- in flux period 3 (Fig. 5a, 5b) may represent compensation by the gill for these losses. The subsequent renewed loss suggests damage to gill tissue which precluded effective compensation, either through permeability changes or transport mechanisms.

In summary, exposure to waterborne Zn^{2+} altered both acid-base and ionoregulation in rainbow trout. Neither the acidemia

nor the ion disturbance alone or in combination appeared sufficient to cause the observed mortality within the time period of the experiment. As well, the Pa_{O_2} was unaffected. The primary lethal mechanism may well operate at the cellular level with the most likely effects either on oxygen delivery and/or utilization, or calcium homeostasis.

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Resource Partitioning and Niche Shifts of Bream (*Abramis brama*) and Eel (*Anguilla anguilla*) Mediated by Predation of Smelt (*Osmerus eperlanus*) on *Daphnia hyalina*

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The resource partitioning of the bream (*Abramis brama*) and eel (*Anguilla anguilla*) populations in Lake Tjeukemeer, The Netherlands, was related to the variation in abundance of their most important food organisms, *Daphnia hyalina* and larval chironomids. Niche shifts of both bream and eel populations were related to the abundance of young planktivorous fish, particularly smelt (*Osmerus eperlanus*). When these fish were abundant the *D. hyalina* population was dominated by small individuals and bream switched from a planktivorous to a benthivorous diet, the condition of mature bream deteriorated, and its gonads developed poorly. Under these circumstances the eel population switched from a diet of chironomid pupae and molluscs to one of predominantly fish. The condition of eels smaller than 35 cm decreased and the chironomid population decreased in numbers and biomass. Conversely, when the recruitment of planktivorous fish was poor, the size of *D. hyalina* was large and diets and conditions of bream and eel populations changed again. Because of the hydrological regime the stock of the young planktivorous fish is determined to a great extent by the immigration of allochthonous larval smelt and varies markedly, but the biomass of the bream and eel populations is comparatively stable.

Le compartimentage des ressources chez les populations de brème (*Abramis brama*) et d'anguille (*Anguilla anguilla*) du lac Tjeukemeer (Pays-Bas) est lié à la variation de l'abondance de *Daphnia hyalina* et des larves de chironomidés, leurs plus importantes proies. Les modifications de la niche chez ces poissons étaient en rapport avec le nombre de jeunes poissons planctivores, en particulier l'éperlan (*Osmerus eperlanus*). Quand des derniers étaient abondants, la population de *D. hyalina* était surtout composée de petits individus; la brème, qui était planctivore, est donc devenue benthivore, la condition des brèmes matures s'est détériorée et leurs gonades se sont mal développées. Dans ces circonstances, l'anguille a remplacé son régime de pupes de chironomidés et de mollusques par un régime composé totalement de poissons. La condition des anguilles de taille inférieure à 35 cm s'est détériorée tandis que la population de chironomidés subissait une baisse en nombre et en biomasse. Inversement, quand le recrutement de poissons planctivores était faible, la taille de *D. hyalina* était élevée tandis que le régime et la condition des populations de brème et d'anguille changeaient à nouveau. À cause du régime hydrologique, l'importance du stock de jeunes poissons planctivores est déterminée en grande partie par l'immigration de larves d'éperlan allochtones et elle varie nettement, quoique la biomasse des populations de brème et d'anguille soit relativement stable.

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Resource partitioning is generally regarded as a means to realize the coexistence of different species (Hutchinson 1959; MacArthur 1972; Schoener 1974). The process of resource partitioning is often considered as very dynamic, depending not only on the functional morphology of the organisms, but also on the interaction between the different species. Studies by Werner (1977), Werner and Hall (1976, 1977, 1979), Nilsson (1960, 1963, 1978), Zaret and Rand (1971), and Magnan and Fitzgerald (1982, 1984) showed that niches are occupied differently when species live sympatrically or allopatrically. This phenomenon of niche shift is commonly observed in lean periods (Schoener 1982). The mechanism of this interaction is generally interpreted as competition, in which interference or depletion of available resources is probably important (MacArthur 1972; Diamond 1978). The introduction of new fish species, which exploit some resources more efficiently than autochthonous species, often causes changes in the structure of food organisms, as was shown in the almost legendary studies of Hrbáček et al. (1961) and Brooks and Dodson (1965), and may cause niche shifts of the autochthonous fish (Nilsson 1978; Magnan and Fitzgerald 1982).

By a coincidence of circumstances a large introduction

experiment is carried out unintentionally in Lake Tjeukemeer every year. During spring, water is pumped from Lake IJsselmeer into the Frisian lake district to maintain a constant water level for agricultural purposes. Together with the water, large numbers of smelt larvae (*Osmerus eperlanus*) are introduced every spring period. The larvae grow up in the Frisian lakes, and being highly efficient planktivorous fish the smelt can affect the zooplankton drastically (van Densen and Vijverberg 1982), but no permanent population can develop here, since the smelt have almost disappeared in spring of the next year because of predation by pike-perch (*Stizostedion lucioperca*) (van Densen and Vijverberg 1982).

In this study we attempted to show that the resource partitioning of bream (*Abramis brama*) and eel (*Anguilla anguilla*) in Tjeukemeer is largely affected by a varying predation pressure on the most abundant food organisms, *Daphnia hyalina* and larval chironomids, because of great fluctuations in the yearly recruitment of planktivorous smelt. In order to explain this mechanism we monitored not only diets but concurrently prey abundances, as well as condition and density of fish per length class.

Study Area

Tjeukemeer is a very shallow (mean depth 1.5 m), hypertrophic, and turbid (Secchi disc depth 25–35 cm) Netherlands freshwater lake with a surface area of 21.5 km^2. The inshore zone is poorly developed, the index of shoreline development being 1.25 (Hutchinson 1957). Reeds provide the only vegetation. Because the lake is shallow and exposed to wind, summer stratification does not occur, and as a result, zooplankton is almost homogenously distributed (de Nie et al. 1980). The lake is part of an interconnected system of lakes that receives water from the nearby Lake IJsselmeer during the summer and from the surrounding polders in winter, when precipitation is larger than evaporation. The management of the hydrological regime is designed to keep a constant water level and is needed for the surrounding agricultural country. For chemistry and hydrology see de Haan (1982) and Leenen (1982).

Materials and Methods

Fish, zooplankton, and chironomid larvae were collected simultaneously during five years (1979–1983), except eels which were only collected during three years (1979–81). The fish were caught once a month with a small mesh trawl (5.5 mm cod end) at five stations. It was difficult to collect eels in sufficient numbers in daytime when they are burrowed in the bottom sediments. These fish were therefore collected at approximately sunset, when they were actively feeding. When fishing at night, we were restricted to only two sites. Thus, comparison of eel and bream data was based on different numbers of sampling sites. However, differences in diet (χ^2 test, $p > 0.8$) and condition (ANOVA, $p > 0.9$) of bream from different sites were not significant. The length of the fishes was measured (fork length) and bream of length classes 9.5–10.5, ..., 34.5–35.5 cm and eels of length classes 12.5–17.5, ..., 32.5–37.5 cm were retained for analysis. At least five fishes per length class were analyzed further. The fishes were measured to the nearest millimetre, weighed to the nearest 0.1 g fresh weight, and sexed and their gonad weights measured. Length–weight relationships for calculating total weight and gonad weight were determined using geometrical functional regressions following Ricker (1973). Condition factors were calculated for the different length classes using the Fulton index, $F = W/L^3 \times 100$, in which W is grams fresh weight and L is length in centimetres (Ricker 1975).

Gut contents were lumped per length class and preserved in 4% formalin. Three or four subsamples were taken per sample and analyzed microscopically to identify, count, and measure food organisms. The length of the consumed chironomids was reconstructed using the size of the head capsules, which are indicative of the original size of the larvae (Lasker 1948; J. Vijverberg, unpubl. results). Energy contents were calculated using the relationships for zooplankton (Vijverberg and Frank 1976), chironomid larvae (Laskar 1948; J. Vijverberg, unpubl. results), and gammarids (Chambers 1977). For chironomids, 1 g ash-free dry weight was assumed to represent 20.9 J (Winberg et al. 1971). The mutilated and thus unrecognizable prey fish were identified and sized by their otoliths (W. L. T. van Densen, unpubl. results).

Zooplankton was sampled with a 5-L Friedinger sampler with a cylinder of 60 cm height. At each of the five stations, two samples were taken, one just below the water surface and the other just above the bottom. The samples were concentrated by filtering through a 120-μm-mesh plankton gauze and preserved in 4% formalin. The combined sample was subsampled with a whirling vessel after Kott (1953) and organisms were identified, counted, and their lengths measured. The length of the cladocerans was measured from the top of the head or, if a helmet was present, from the base of the helmet to the base of the tail spine.

For sediment, 16 bottom samples were taken with a modified Ekman–Birge grab (Beattie 1979) or Petterson grab, depending on the nature of the bottom substrate. Ten samples were taken in duplicate in a sandy transect from the shore to the middle of the lake at 5, 100, 500, 1000, and 1500 m from shore. Similarly, six samples were taken in a muddy transect at 5, 100, and 500 m from shore. The samples were sieved through a 0.5-mm sieve using tap water with a shower appliance; the larvae were preserved in 4% formalin and subsequently identified, counted, and measured. The length was measured from the top of the head capsules to the end of the tail. Per station, the areal density (per square metre) and biomass were calculated and the average density was computed by accounting for the relative area of different bottom substrates (Beattie 1982).

Results

In mature bream (≥30 cm in Tjeukemeer), somatic and gonad growth did not occur simultaneously. Somatic growth started just after spawning and continued until late summer. In this period, condition recovery and length growth occurred if feeding conditions were good. In late summer, gonad development started and continued until spawning in spring. In the five study years, both the somatic and gonad growth fluctuated strongly together with the density and biomass of the most important food organisms, zooplankton and chironomids.

Spawning usually occurred in May, but in 1982 it was delayed until June. The recovery from spawning proceeded very well in 1979 and 1983, when the total weight of 35-cm female bream reached maximal values of about 750 g fresh weight (Fig. 1). During the three summers in between, recovery was not complete since more weight was lost by spawning than was gained by recovery, leading to condition deterioration in the course of these years (1980–82). Only in late summer of 1982 was recovery strong, this amelioration of condition continuing

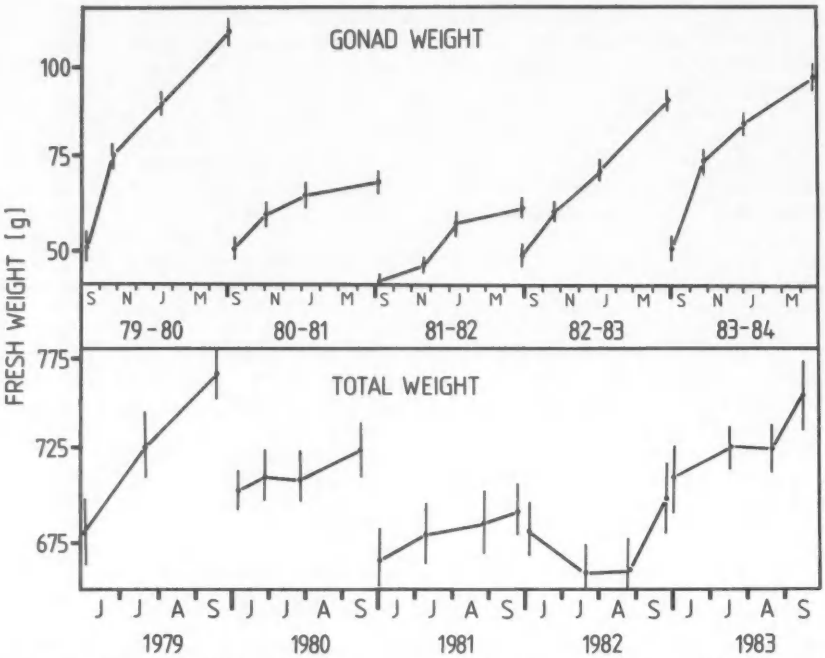

FIG. 1. Fresh weight of 35-cm female bream (with SE) during the summer months of five years and gonad weight (with SE) after these summer months.

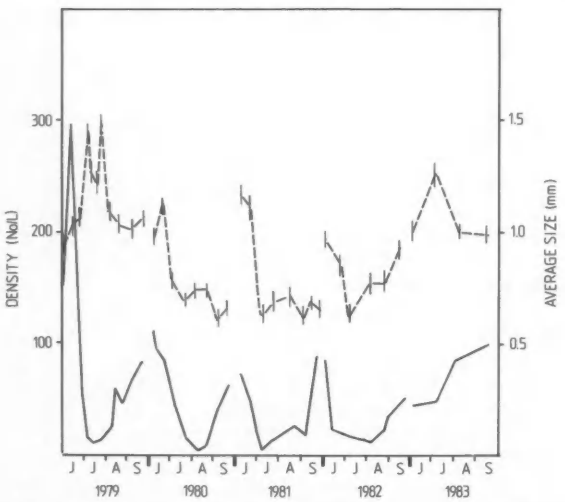

FIG. 2. Density (solid lines) and average size of *D. hyalina* (with SE) (broken lines) during the summer months of five successive years.

in 1983. Accompanying this condition recovery, gonad development proceeded well after the summers of 1979, 1982, and 1983, but was poor in 1980 and 1981 (Fig. 1). The latter reflected a deterioration of feeding conditions in 1980 and 1981 and an improvement during 1982.

Feeding Conditions and Food Composition

The most important food organisms for mature bream were large zooplankton, *D. hyalina* and *Leptodora kindtii*, and larval chironomids (≥6 mm), namely *Polypedilum nubeculosum*, *Einfeldia carbonaria*, and *Chironomus plumosus*. The density and size composition of *D. hyalina* fluctuated strongly both seasonally and yearly during the study period. This was also true for the density and biomass of chironomids (≥6 mm).

The density of *D. hyalina* was generally highest in June, i.e. 100–300 individuals/L. This was followed by a sudden decrease to about 10–20 individuals/L in July (Fig. 2). In 1983 only the summer maximum had probably already occurred in May. The recovery of this collapse had already started in August of 1979 and 1983 but not until September of 1980, 1981, and 1982. The difference in density between years was only significant (Kruskal–Wallis analysis of variance, $p < 0.05$) for August, but not for the other months.

The average size of *D. hyalina* was also highest in June, with values of 1–1.4 mm (Fig. 2). In 1979 and 1983 these maximum values decreased only slightly to about 1 mm in July and remained at that level during the rest of the summer. In the three years in between, the maximum values decreased to 0.6–0.7 mm and remained at that level in 1980 and 1981. In 1982 the average size increased again to about 1 mm in late summer. The difference in average size of *D. hyalina* between the summers was significant (Kruskal–Wallis, $p < 0.01$). Comparing only the second halfs of the summers the difference was even more pronounced (Kruskal–Wallis, $p < 0.001$).

The density and biomass of the chironomids showed a significant (Kruskal–Wallis, $p < 0.1$) difference between the years. The density fluctuated from 50 to 1200 individuals/m^2 and the biomass from 0.1 to 2.5 g fresh weight/m^2 (Fig. 3). The density and biomass in the summers of 1979 and 1983 were significantly (Kruskal–Wallis, $p < 0.01$) higher than those in 1980 and 1981.

The food of mature bream consisted largely of zooplankton

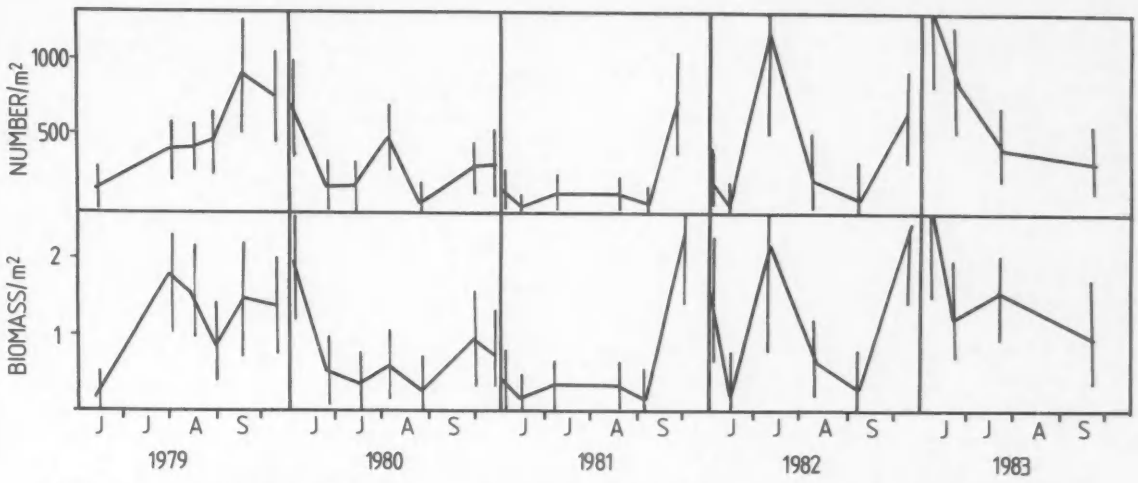

FIG. 3. Density and biomass (with SE) per square metre of chironomid larvae during the summer months of five successive years.

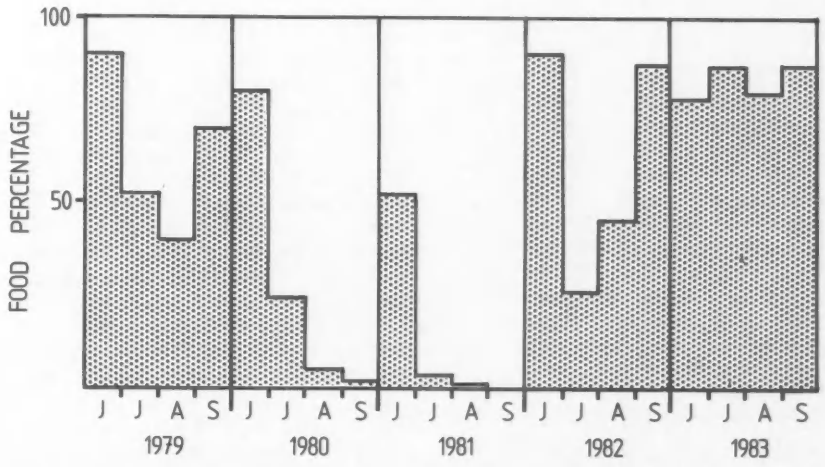

FIG. 4. Percentage food composition based on the energy contents of the food organisms of 35-cm bream during the summer months of five successive years. Stippled areas represent zooplankton; open areas represent chironomid larvae.

and chironomids, with a significant (χ^2 test, $p < 0.01$) variation in their relative amounts between years (Fig. 4). In 1979 and 1983 the share of zooplankton, in particular *D. hyalina* and to lesser extent *L. kindtii*, was relatively large and coincided with a large average size of *D. hyalina* in Tjeukemeer and the good condition and gonad development of bream. In 1980 and 1981, chironomids were the dominant food organisms, although their biomass was very low; condition and gonad development of the fish deteriorated then.

The seasonal variation of the diet showed a more or less similar picture. In June, when the density and average size of *D. hyalina* were high, the major part of the diet was *D. hyalina* (Fig. 4). Usually the fish switched to chironomids in July, when the *D. hyalina* population had collapsed, except in 1983 when there was no collapse at that time. In the second half of the summer, bream switched again to *D. hyalina* if the latter's population had increased in density and size. Thus, the share of *D. hyalina* in the diet was related closely with its average size and density in the lake, but that of the chironomids was inversely related (Spearman rank-test, $p < 0.05$) to its biomass in the lake. This negative correlation points to the (depressing) impact of the mature bream population on the chironomids. The decreasing condition and gonad growth of bream in a period of chironomid depression indicate that the chironomid production was not sufficient to sustain the mature bream. The *D. hyalina* population was, however, sufficient and was hardly depressed by the bream. Its depression in 1980 and 1981 was clearly related to the successful recruitment of smelt and perch (*Perca fluviatilis*) in these years (Fig. 5).

Biomass of Young Planktivorous Fish

The success of the recruitment of young planktivorous fish was greatest in 1980 and 1981 when large amounts of smelt and

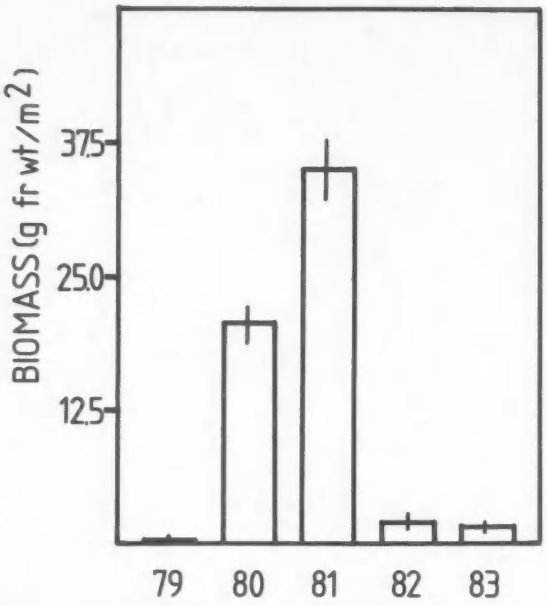

FIG. 5. Biomass of 0+ planktivorous fish (with SE) in September of five successive years.

perch recruited (Fig. 5). Most of the smelt and perch (only in 1980) immigrated passively from nearby Lake IJsselmeer due to the hydrological regime (van Densen and Vijverberg 1982). Together with the water let in during spring from Lake IJsselmeer, large numbers of smelt and perch larvae were transported in the lake district, where they grew up on a zooplankton diet (van Densen 1985). The biomass of these fish at the end of the summer varied significantly (ANOVA, $p < 0.001$) between years (Fig. 5). In 1980 and 1981 the biomass was 200 and 370 kg/ha, while in the other years it was only 5–25 kg/ha.

The successful recruitment changed the availability of *D. hyalina* for mature bream drastically, forced the fish to switch to chironomids, which in its turn resulted in a change in density and biomass of the chironomids. Eel is another important predator of chironomids, but also of fish, if available (de Nie 1982). The changing availability of both chironomids and fish would probably affect the eel population too. In the first three years (1979–81) the diet of the eel population was studied together with its condition and biomass for different length classes. These data were compared with four length classes of the bream from the same period.

Diet of Bream and Eel per Length Class

The food of all length classes of bream studied consisted of zooplankton, benthic cladocerans, and chironomid larvae and differed significantly (χ^2 test, $p < 0.001$) between years and length classes (Table 1). Immature bream (<30 cm) did not feed on chironomids in 1980 and 1981, but on benthic cladocerans such as *Alona* sp. and *Leydigia* sp. These organisms are not available for the mature bream, since they cannot be retained by their gill rakers. In 1979, zooplankton was also dominant in the diet of the immature bream; however, smaller species (*Bosmina coregoni* and *Acanthocyclops robustus*) contributed more than in the mature bream. Only in this year did chironomids contribute to the diet of the immature bream.

The food of the eel consisted largely of chironomids, gammarids, molluscs, and fishes, their proportions in the diet differing significantly (χ^2 test, $p < 0.001$) for different length classes and in different years (Table 1). Smaller eels (<30 cm) consumed relatively more chironomids and gammarids than the larger ones, which ate mainly fish and molluscs. Among the chironomids the large *C. plumosus* was strongly selected; the smaller *P. nubeculosum* and *E. carbonaria* were eaten in relatively smaller numbers. Among the life stages of chironomids the pupae were selected the most, contributing almost 80% of the total chironomid share ((Table 1), whereas the percentage in the sediments was mostly less than 1–2% (J. Vijverberg, unpubl. results). The preference for the pupae could easily be explained by some observations of their feeding behaviour: a foraging eel swims slowly and zig-zagging while touching the bottom with its head. Only larvae or pupae near the surface are noticed, and larvae deep in the sediments rarely. Bream, in contrast, sucks in the bottom substrate and winnows the larvae by sieving and splitting; fine sediment particles disappear between the gill rakers and the coarse particles are spit out. Thus, bream can reach several centimetres deep in the sediments, while eels are restricted to the surface layer, where the pupae are located (Frank 1982). The share of the chironomids in the diet was highest in 1979 when molluscs were the most important alternative food. In the next years the share of chironomids was much lower and was largely replaced by fish as well as by gammarids.

The seasonal variation in diet was significantly (χ^2 test, $p < 0.001$) different for all length classes of bream and eel. Zooplankton was dominant food for bream in June of each year. In the second part of the summers of 1980 and 1981 it was replaced by benthic cladocerans in immature bream and chironomids in mature bream, but in 1979, zooplankton was dominant in both size classes. Eels fed predominantly on chironomids in June of each year. In the second part of the summers of 1980 and 1981 these were largely replaced by fish, but in 1979, chironomids were dominant. The share of chironomids in the diet of the bream and eel populations was significantly (Spearman's rank test, $p < 0.01$) inversely related, weighting the diet of both species for the size distribution of both populations (Fig. 6).

Figure 6 shows a clear difference in chironomid diet of bream and eels between the years for the months of July, August, and September, the share of chironomids being higher in eels than in bream during 1979 and lower in 1980 and 1981. This is hardly reflected in the similarity indices for bream and eels during this period (Table 2); however, it is essential for the understanding of competition. The indices were significantly (Kruskal–Wallis, $p < 0.06$) higher in 1979 for the months of July and August than in the other years. The diet overlap for bream and smelt was calculated in the same way, starting from a 100% zooplankton diet for smelt as an obligate planktivore in its first year (van Densen 1985). The indices were significantly (Kruskal–Wallis, $p < 0.06$) higher in 1979 for the months of August and September than in the other years. Thus, in the year when food resources were highest (1979), diet overlaps were highest.

Condition, Size Distribution, and Density of Bream and Eel Populations

The condition of bream ≥ 30 cm varied significantly (ANOVA, $p < 0.01$) between years, while the immature bream hardly differed (ANOVA, $p > 0.5$) (Fig. 7). The fish recovered from spawning very well in 1979, but almost failed to do so in the other years. The immature bream did not differ in growth

TABLE 1. Percentual food composition based on energy contents of food organisms of four size classes of bream and eel during three successive summers (1979–81) in Tjeukemeer (width of the bream length classes is 19.3–20.7, 24.3–25.7, etc., and of the eel length classes is 17.5–22.4, 22.5–27.4, etc.).

	Length class (cm)											
	1979				1980				1981			
	20	25	30	35	20	25	30	35	20	25	30	35
Bream												
Zooplankton	76	68	65	61	25	24	30	25	20	25	15	15
Benthic cladocerans	2	2	1		65	51	5		75	60	20	2
Chironomid larvae	21	30	33	39	10	25	64	74	5	15	64	83
Chironomid pupae	1		1				1	1			1	
Eel												
Fish		6	13	28	7	30	62	80		50	86	97
Molluscs	5	22	41	42	1		5	7		2		
Chironomid larvae	21	19	12	6	9	8	9	3	20	10	3	1
Chironomid pupae	74	53	34	14	71	54	21	8	75	32	9	2
Gammarids					13	8	3	2	5	6	2	

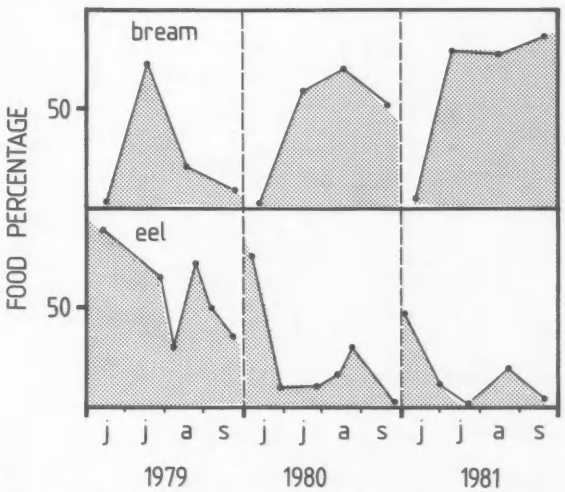

FIG. 6. Share of chironomids (stippled) based on their energy contents in the diet of the bream and eel populations. The diet is a weighted mean for both populations in the length range 15–40 cm.

response to the change in diet. The condition of eels ≤30 cm was significantly (ANOVA, $p < 0.01$ for 25 and 30 cm; $p < 0.05$ for 20 cm) higher in the first half of the three years (Fig. 8). Apparently the availability of fish could not compensate for the loss of chironomids. The condition of larger eels did not significantly (ANOVA, $p > 0.5$) differ in the two periods, but its condition was significantly (ANOVA, $p < 0.001$) higher than that of 30 cm eels. Larger eels can apparently handle smelt with a size of 4–7 cm in August–September easily.

The part of the bream population feeding on chironomids fluctuated in 1979–81 between 400 and 500 individuals/ha, with a corresponding biomass of 200–250 kg/ha (Table 3). These are minimum estimates, as the efficiency of the trawl is based on catches of small 0+ fish with known densities determined with a purse seine. Using this estimate of the bream biomass the daily energy requirements for maintenance metabolism can be calculated and compared with the amount available.

TABLE 2. Schoener's (1970) similarity index for the diet of bream and eel (weighted for the whole population) and for that of bream and smelt in the summer months of 1979–81.

	1979	1980	1981
Bream–eel			
June	5	4	4
July	75	24	9
August	25	21	19
September	10	16	10
Smelt–bream			
June	94	90	85
July	23	35	15
August	72	15	15
September	87	30	10

Using the average water temperature of 17–18°C in the summers of 1980 and 1981 in Winberg's (1956) basic equation the daily maintenance energy requirement for this part of the bream population would be 0.8% of its biomass, i.e. 0.16–0.2 × 1.25 g fresh weight chironomids·m^{-2}·d^{-1}. As the condition decreased the bream ate less per day. Otherwise it would have consumed about 50% of the total chironomid biomass daily (0.2–0.5 g fresh weight/m^2); such a consumption is not realistic, because a chironomid production of 50% per day is impossible (MacKay 1977; Waters 1979; Beattie 1982; Wilda 1984).

The same calculations can also be made for the eel population that fed on chironomids with a biomass of 20–25 kg/ha (Table 2). The daily maintenance metabolism of this part of the eel population at 17–18°C would require 0.03–0.04 × 1.25 g fresh weight chironomids·m^{-2}·d^{-1}. This amount is 10% of the total chironomid biomass and could not apparently be collected as the condition of the eels decreased. We know that the pupae of chironomids were highly preferred to larvae by eel, and considering the chironomid biomass it would mean that almost 10% of the chironomids should be pupae to meet the requirements of the eel. The percentage of pupae was maximally 1–2% and thus not sufficient.

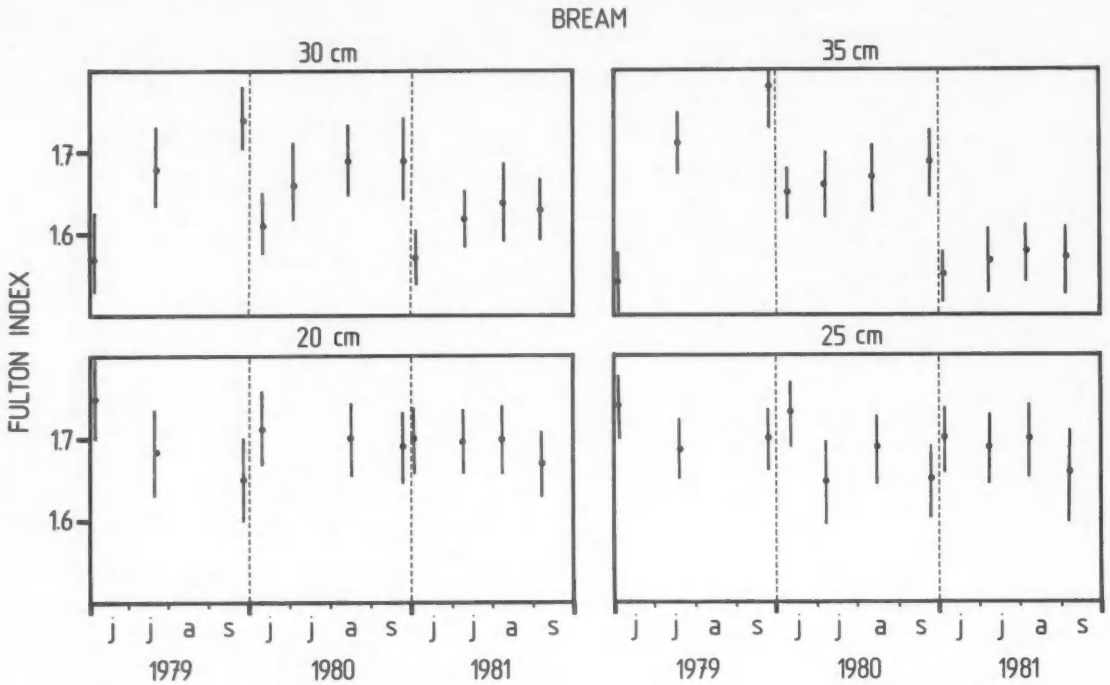

FIG. 7. Condition of four length classes of bream (with SE) in the summer months of 1979–81.

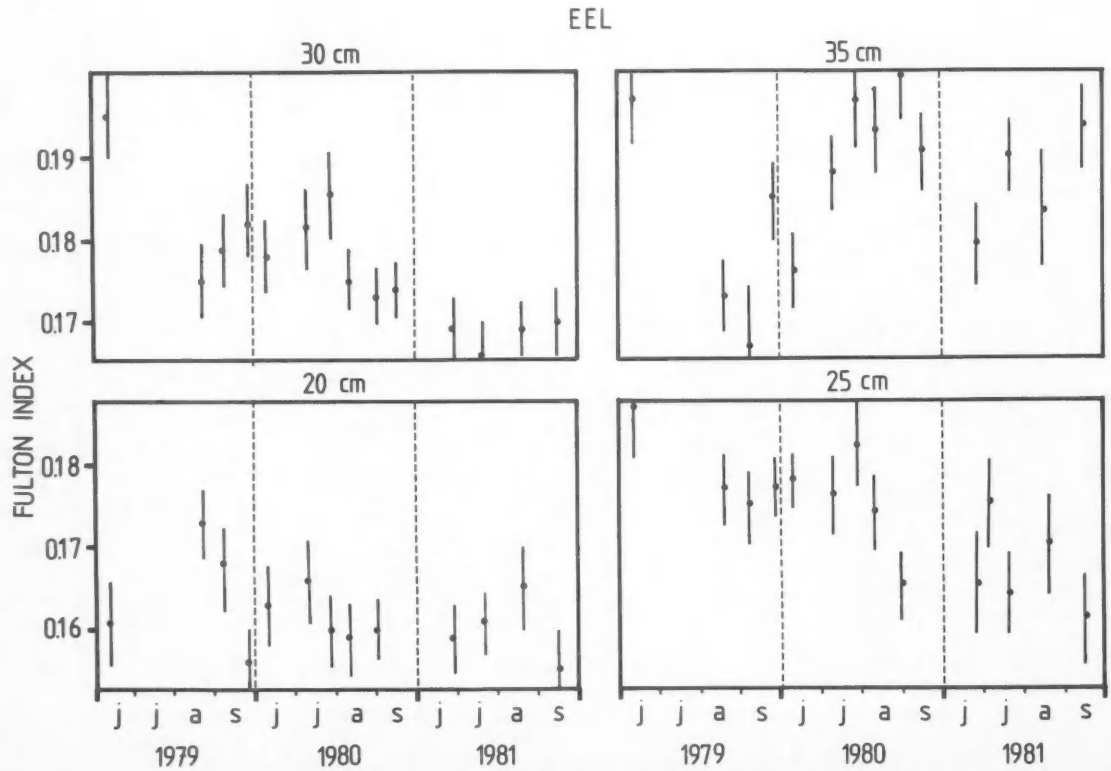

FIG. 8. Condition of four length classes of eel (with SE) in the summer months of 1979–81.

TABLE 3. Average density (individuals) and biomass (kg) per hectare (±SE) of five length classes of bream and eel in 1979, 1980, and 1981.

	Length class (cm)				
	15–20	20–25	25–30	30–35	35–40
Bream density					
1979	31±6	243±51	212±45	148±39	25±11
1980	28±7	122±29	276±52	186±45	35±13
1981	67±20	31±8	276±55	201±49	39±14
Bream biomass					
1979	3±1	45±9	76±16	87±23	22±10
1980	3±1	24±6	97±18	106±26	30±11
1981	6±2	6±2	95±19	112±27	32±11
Eel density					
1979	158±24	346±87	298±60	149±42	40±12
1980	401±68	444±93	495±109	192±58	46±14
1981	460±87	476±138	325±78	169±54	94±30
Eel biomass					
1979	2±1	7±2	11±2	9±2	4±1
1980	4±1	8±2	18±4	11±3	4±1
1981	4±1	9±3	11±3	10±3	9±3

Discussion

Resource partitioning is a generally accepted phenomenon and usually regarded as a mechanism to realize the coexistence of different species (Hutchinson 1959; Schoener 1974; MacArthur 1972). Partition of resources is mostly determined by the utilization efficiencies or functional morphology of the coexisting species (Werner 1977, 1979; Laughlin and Werner 1980; Mittelbach 1984; Nilsson 1978; Magnan and Fitzgerald 1984). Several studies (see Schoener 1982), however, show that overlaps in diet are highly variable; in periods of plentiful resources the overlap is considerable, but in lean periods overlaps are minimal. Our study showed that overlaps were highest when resources were abundant and condition of fish was good. A deterioration of condition and low availability of food resources was accompanied by niche shifts of bream and eel and a consequently smaller diet overlap. Diamond (1978) stated that although niche shifts are the best evidence for interspecific competition, the mechanisms for this phenomenon, which may be fighting or lowering of resource levels, have never been demonstrated. He stated: "until resource levels are actually measured an essential link in the argument remains hypothetical and the 'overwhelming' evidence for competition remains circumstantial." We have made this link and have given evidence that competition occurs through lowering of resource levels.

The studies of Nilsson (1960, 1963) and Zaret and Rand (1971) were the first to show that resource partitioning in fishes is maximal in periods when food is scarce. Nilsson (1978) suggested that the mechanism of this niche shift, which he called interactive segregation, was largely determined by a change in the structure of the size distribution of the food organisms due to size selective predation. He showed that diets and availability of food organisms were different when trout, Arctic char (*Salvelinus alpinus*), and whitefish (*Coregonus* sp.) were allopatric or sympatric and he suggested that the availability of food organisms was determined by the most efficient predator. In a more or less similar way, Werner and Hall (1976, 1977, 1979) showed differences in diet for sympatric and allopatric Centrarchidae. The phenomenon of niche shift was also found by Magnan and FitzGerald (1982, 1984) and Post and Cucin (1984) after the introduction of a new fish species that was better adapted to exploit some food organisms than the autochthonous species and caused changes in the composition of the benthic community of food organisms. This impact of introduced or removed fish species on the structure of food organisms was also shown by Brooks and Dodson (1965), Hrbáček et al. (1961), Lynch (1979), Nilsson and Pejler (1973), de Bernardi and Giussani (1975), Drenner et al. (1982), and many others for zooplankton, whereas Ball and Haynes (1952), Hall et al. (1970), Andersson et al. (1978), Crowder and Cooper (1982), and Post and Cucin (1984) showed this for the impact on zoobenthos.

We have shown that the diet shifts of bream and eel were clearly caused by the change in availability of *D. hyalina* and larval chironomids, which in turn were related to the abundance of smelt: a high biomass of smelt reduced the availability of *D. hyalina* for mature bream and forced the latter to switch to chironomids, which were also depressed, affecting the diet of the eel. The young smelt are efficient planktivorous fish that affect the size structure of the zooplankton community easily by their size selective predation (van Densen 1985). When the density of smelt is very high, the depression of the average size of *D. hyalina* is so drastic that these food organisms can no longer be retained by the gill rakers of mature bream (Lammens 1985), which has to switch to a benthivorous diet. When feeding on benthos, the bream takes mouthfuls of bottom substratum and winnows the chironomid larvae by sieving and spitting. By this feeding behaviour, bream has access to chironomids several centimetres deep in the substratum. Eels in contrast can only forage efficiently in the top layer of the substratum and are therefore restricted to the chironomid pupae. Feeding predominantly on chironomids the large bream population decreases the availability of pupae to such an extent that eels are forced to switch to another diet. The same fish that indirectly cause this switch become a main food item for eels >25 cm. Although the density of smelt was very high in 1980 and 1981, the real availability for eels ≤30 cm appears to be too small, probably because these smelt are too large for these eels to handle.

The condition and gonad development of the mature bream showed that *D. hyalina* could sustain the bream population unless it was depressed by smelt. The chironomid population was clearly not sufficient for this large bream stock. This situation in which the bream population is largely dependent on large zooplankton seems to be a rare situation in European inland waters, since usually, chironomids contribute the greatest part to the diet and growth of the bream population (Laskar 1948; Zadorozhnaya 1977; Prejs 1976) and cladocerans are only important for the smaller bream (Kokes and Gajdusek 1978; Laskar 1948) or only incidentally for the larger ones (Kogan 1970; Zadorozhnaya 1977). It is not clear why in these cases the larger cladocerans are not available for longer periods. Partitioning of resources is probably maximal here, and the zooplankton niche is probably always occupied by a more efficient planktivorous fish, or the production of large zooplankton is limited by other causes.

The eel population is largely dependent on chironomid pupae and to a lesser extent on fish and molluscs. The importance of these chironomids is also clear in most other studies, but isopods, trichopterans, and ephemeropterans are preferred, if available (Frost 1946; Burnet 1952; Sinha and Jones 1967; Moriarty 1979; Rasmussen and Therkildsen 1979). These organisms, however, are hardly present in the open water of Tjeukemeer and therefore not found in the stomachs. In the open water of Lake Balaton, chironomids are also the main food item for the eel (Biro 1974). Fish are seldom found in eels ≤ 30 cm, only in the larger individuals (Frost 1946; Opuszýnski and Leszczýnski 1967; Micheler 1967). In Tjeukemeer this only occurs when the availability of chironomids is reduced because of the recruitment of exceptionally high numbers of smelt, which become the major food for the eel.

Our study shows that interspecific competition in Tjeukemeer is mediated by reduction of the availability of the common food resources by the most efficient predators. Thus, predation here is the motor of interspecific competition. It remains, however, far from clear whether this competition leading to resource partitioning realizes the coexistence of these species. Since bream and eel are very well protected against predation (by pike-perch) because of their size and burrowing behaviour, respectively, they can reach the carrying capacity of the system and partition resources. The other species such as perch, ruff (*Gymnocephalus cernua*), roach (*Rutilus rutilus*), and white bream (*Blicca bjorkna*) occur in much lower densities (Vijverberg and van Densen 1984), mostly in the periphery of the lake, and are all vulnerable to predation because of their size and shape, and probably only find some refuge in the littoral region. The studies of Mittelbach (1981) and Werner et al. (1983) have stressed the importance of refuges for fishes. This is not only important for the distribution of the size classes within a species, as they demonstrated, but probably also for the distribution of the smaller species within a lake. Feeding conditions in the open water may, however, be too poor for the other cyprinids which prefer larger food organisms than bream (Lammens 1984).

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Tidal Exchange of Anadromous Rainbow Smelt (*Osmerus mordax*) Larvae Between a Shallow Spawning Tributary and the St. Lawrence Estuary[1]

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Ouellet, P., and J. J. Dodson. 1985. Tidal exchange of anadromous rainbow smelt (*Osmerus mordax*) larvae between a shallow tributary and the St. Lawrence estuary. Can. J. Fish. Aquat. Sci. 42: 1352–1358.

The factors involved in the riverine transport and tidal exchange of yolk-sac rainbow smelt (*Osmerus mordax*) larvae between Ouelle River and the St. Lawrence estuary were documented. A 72-h sampling series provided evidence of synchronous nighttime hatching of larvae independent of water temperature and river hydrodynamics. Although light conditions influence the vertical distribution of yolk-sac larvae at the river's mouth, the influence of current speed on larval vertical distribution appears to be a more important element in the dynamics of riverine drift and tidal exchange. Yolk-sac larvae sink to the bottom as water currents decrease prior to low slack water. With the intrusion of estuarine water along the bottom during the flood, yolk-sac larvae drifting from the river appear to be mixed with larger post yolk-sac larvae of estuarine origin resulting in temporary retention at the mouth of Ouelle River. However, longer ebb phases in the river result in a net transport of smelt larvae towards the St. Lawrence estuary. We conclude that the riverine transport and mixing of yolk-sac larvae with the estuarine water mass is controlled principally by the hydrodynamics of the system and the nighttime hatching and specific gravity of yolk-sac larvae.

Les facteurs impliqués dans le transport en rivière et le passage à l'estuaire des larves d'éperlan (*Osmerus mordax*) ont été étudiés à la rivière Ouelle dans l'estuaire moyen du Saint-Laurent. L'échantillonnage a révélé que l'éclosion des larves était synchrone, de nuit, indépendamment de la température et de l'hydrologie de la rivière. Malgré que l'intensité lumineuse affecte la distribution verticale des jeunes larves, la vitesse du courant, dans la rivière, semble être un facteur plus important dans le contrôle de la dynamique du transport. Les larves vésiculées coulent et se retrouvent près du fond lorsque le courant ralenti, dans la rivière, avant l'étale de la marée basse. A l'introduction de la masse d'eau estuarienne sur le fond, au montant, les larves dérivant dans la rivière seraient ainsi mélangées à des groupes de larves plus âgées, provenant de l'estuaire, indiquant une rétention temporaire des larves près de l'embouchure. Toutefois, le transport net est dirigé vers l'estuaire dû à la longue durée des courants baissants dans la rivière. En conclusion, le transport en rivière et le mélange des larves vésiculées d'éperlan aux eaux de l'estuaire semblent être principalement sous le contrôle de; l'hydrodynamique du système, l'éclosion nocturne et de la densité spécifique des larves à sac vitellin.

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Anadromous rainbow smelt (*Osmerus mordax*) larvae, one of the principal components of the ichthyoplankton community of the St. Lawrence estuary, are most abundant in the partially mixed northern portion of the middle estuary upstream of Ile aux Coudres (Fig. 1) where they are associated with the fresher and warmer upstream water masses (Able 1978; Courtois et al. 1982; Ouellet and Dodson 1985). Anadromous smelt reproduce in several south shore tributaries of the St. Lawrence estuary, the most important being Boyer and Ouelle rivers (Fig. 1), where they generally spawn above the upstream limit of tidal incursion following spring breakup (Fréchet et al. 1983).

The vertical and horizontal distribution of anadromous smelt larvae from hatching in the Boyer River to their occupation of nursery areas in the middle estuary was documented in 1982 to describe the mechanisms contributing to the apparent retention of smelt larvae in this area (Ouellet and Dodson 1985). We observed peaks of larval abundance downstream of spawning grounds at night indicating a 24-h periodicity in hatching and the introduction of larvae into the riverine circulation. The entire hatching and riverine drift sequence was completed in approximately 12 d and no retention of larvae was observed over the 2 km separating the spawning ground and river's mouth.

The distribution of smelt larvae at the mouth of the Boyer River was characterized by the apparent daytime aggregation of larvae followed by the movement of patches of larvae into the St. Lawrence estuarine water mass every 24 h at night following high tide (Ouellet and Dodson 1985). However, the large-scale sampling program used to describe the seasonal distribution patterns of smelt larvae in 1982 was not precise enough to

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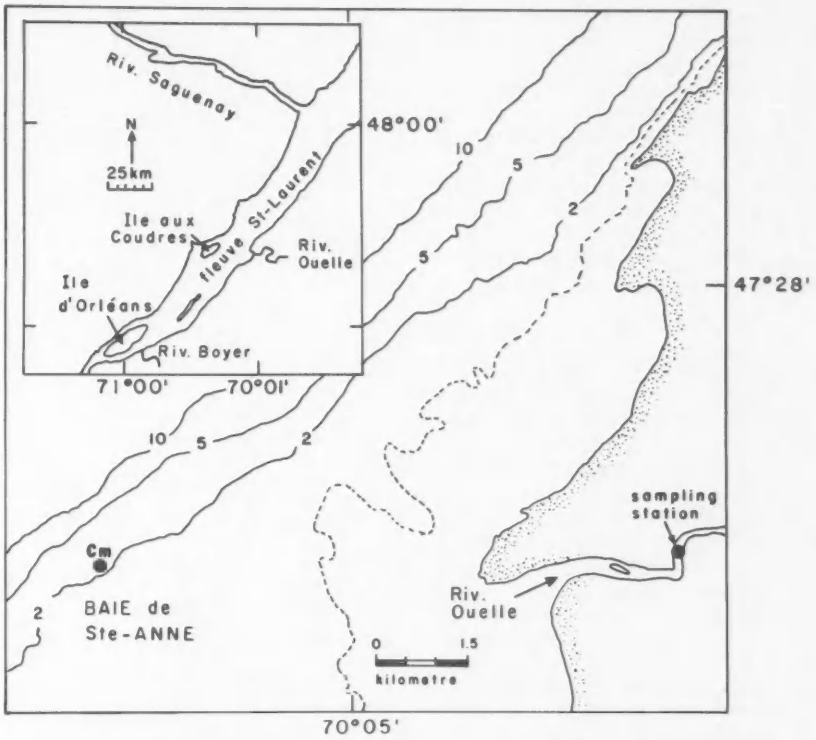

FIG. 1. Study area and location of currentmeter stations and smelt larvae sampling station in Ouelle River. Isobaths are in metres and broken line indicates lower limit of low water.

determine the mechanism controlling the apparent accumulation and flushing of smelt larvae. We hypothesized that the daytime accumulation resulted from the tendency of negatively buoyant yolk-sac larvae to sink to the bottom at low current speeds and to remain there during daylight due to the negative phototaxis exhibited by small smelt larvae. We further proposed that during dusk and darkness, larvae rise from the river bed, thus contributing to the 24-h periodicity in larval flushing observed following high tides at the river's mouth. The objectives of the present study were to document the factors involved in the exchange of yolk-sac smelt larvae between Ouelle River and the St. Lawrence middle estuary and to test the hypothesis that active phototaxic behavior of recently hatched yolk-sac larvae contributes to regulating the initial stages of larval drift.

Materials and Methods

Study Area and Physical Assessment

Ouelle River is a shallow and meandering river that flows into the southeast end of Baie de Ste-Anne (Fig. 1), a broad shallow platform extending from the south shore of the St. Lawrence estuary to the edge of the deep south channel. At the sampling station (Fig. 1), the river is 100 m wide and has a mean depth of 1.5 m at low water. Tidal amplitudes at the station varied from 2.6 m for the highest high tides to 1.5 m for the lowest low tides. Tidal influence was estimated to extend 7 km upstream during spring runoff in May 1983. Geophysical characteristics and the dominant water circulation features of the Baie de Ste-Anne area are provided by d'Anglejan et al. (1981).

The field program began on 3 May 1983 following ice breakup in Ouelle River and continued for 1 mo. To characterize the tidal current in the estuarine channel of the river and in the adjacent Baie de Ste-Anne region, two Aanderaa water currentmeters were moored at selected stations in each site (Fig. 1). Currentmeters were positioned at a height of 1 m above the bottom and set to record simultaneous data from the river's mouth and Baie de Ste-Anne. The recording period, between 5 and 30 May 1983, monitored the water circulation in the area for both spring and neap-tide cycles.

Estimation of river discharge and calculation of material fluxes from a river require detailed measurements of water flow across the river cross-section. Simultaneous profiles of velocity, temperature, and salinity (VTS) recorded at stations located on a river cross-section adequately represent cross-sectional mean VTS values (Kjerfve et al. 1981). The cross-section occupied at the river's mouth (Fig. 1) was divided into three subsections positioned at particular points related to the local bathymetry (Kjerfve et al. 1981). However, due to technical constraints, sequential VTS profiles were recorded rather than simultaneous ones. Nevertheless, the river is not wide (100 m at high water) permitting rapid displacement between subsections, so it is quite likely that the consequences were minimal on the estimation of VTS mean values. Physical variables were recorded at 1-m intervals on each profile using a Marsh–McBirney portable currentmeter coupled with thermo-salinometer sensors.

Smelt Larvae Sampling

Sampling of smelt larvae was conducted at the river station

(Fig. 1). Two discrete depths of the water column were sampled so as to cover the river cross-sectional area. A 0.5-m standard conical plankton net equipped with TSK flowmeter and fitted with a No. 6 (0.250 mm) mesh net was used for the surface horizontal tow. The same apparatus mounted on a suprabenthic sled (Henri et al. 1985) was used to sample the bottom layer of the water column. The sampling schedule consisted of a 72-h series (28–31 May 1983) where the bottom and the surface layers were successively sampled for smelt larvae followed by the measurement of VTS profiles at each subsection. This sequence of two 5-min plankton net samples and flow measurements was repeated every hour for the 72-h period.

Analysis

All samples were preserved in 4% formalin. Samples containing more than 200 smelt larvae were subsampled using a Folsam partitioner; samples containing less than 200 smelt were completely sorted. A maximum of 50 smelt larvae from each sample were arbitrarily chosen and measured to the nearest 0.05 mm.

Water current data from Ouelle River was analyzed to reveal significant periodicities in water circulation using harmonic analysis (Legendre and Legendre 1979). The periodogram produced reveals the percentage of the total variance accounted for by a given period of the water current series. The variance estimates of the harmonic analysis were calculated according to Thrall and Engelman (1983).

A contingency table analysis was performed to determine the contribution of light conditions and river current speed on the vertical distribution of yolk-sac smelt larvae in the river water column. It is also possible to test a posteriori if an observed value (O) in the table significantly differs from the associated expected value (E). A test value is calculated with the expression

$$\frac{|O - E|}{\sqrt{O(1 - O/p)}}$$

where p represents the total number of observations in the contingency table. If the result obtained was greater than the value $(1 - 2\alpha/rc)$, where $\alpha = 0.05$ and represents the probability level of significance, r represents the number of rows, and c represents the number of columns in the contingency table, we concluded that the observed value is significantly greater than the expected value.

Larval Flux

The hourly estimates of larval smelt flux (number per second) were calculated for both surface and bottom layers as the concentration of larvae (number per cubic metre) multiplied by the river flow estimated at the moment of sampling (cubic metres per second) in each layer. The depth of the surface layer was 1 m and the depth of the bottom layer was measured from the bottom to 1 m below the surface. The total number of larvae passing the river's mouth in surface and bottom layers during any given tidal state was estimated by multiplying the mean concentration of larvae in each layer for each tidal state by the volume of water ebbed or flooded in each layer (Johnson and Conor 1982).

Results

Hydrodynamics

The tidal current speed curves (Fig. 2) revealed that although

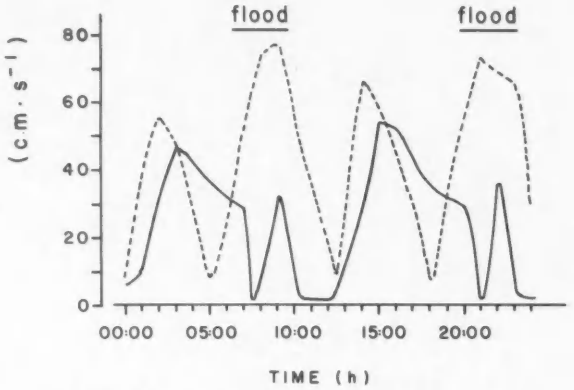

FIG. 2. Comparison of ebb and flood current velocity curves between Ouelle River (—) and Baie de Ste-Anne (---) for a typical 24-h sequence.

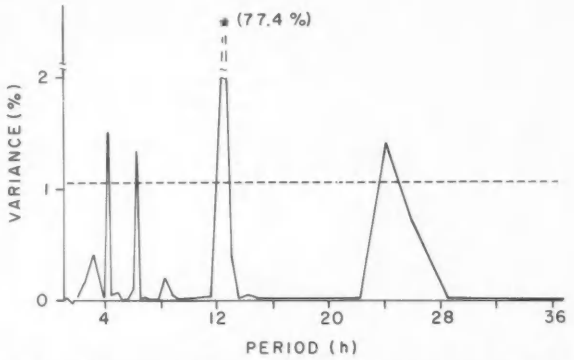

FIG. 3. Schuster periodogram of current data at the Ouelle River sampling station for May 1983. Horizontal broken line indicates critical value at $p = 0.05$.

the ebb and flood phases in Baie de Ste-Anne are approximately of equal duration, the ebb phase in Ouelle River is longer than the flood. Due to this phase difference, the ebbing river water is caught by the incoming flood water and thus retained in the bay area. The harmonic analysis performed on the water current data recorded in the river for the period of May 1983 revealed that water circulation is dominated by the tidal cycle (the 12.5-h period, Fig. 3) and two shorter cycles (the 6-h and 4-h periods, Fig. 3). These periods most probably represent the differences observed in the tidal speed curve at the river station (Fig. 2), the ebbing phase (the 6-h period) being longer than the flood (the 4-h period).

Water temperature and salinity in the river fluctuated with the tidal cycle (Fig. 4). Salinity was recorded in the river only at high tides and was associated with the cold water mass coming from the estuary. The sampling station was situated near the upstream limit of salt intrusion in the river; thus, the lowest high tide observed during the series (hours 36–38) was too weak to force the estuarine salt water upstream to the station (Fig. 4). Water temperature progressively increased during the daytime ebbing flow (Fig. 4), indicating a longitudinal gradient of water temperature in the river. At night, the temperature of the ebbing river water decreased progressively. Atmospheric conditions also influenced the evolution of water temperature in the river.

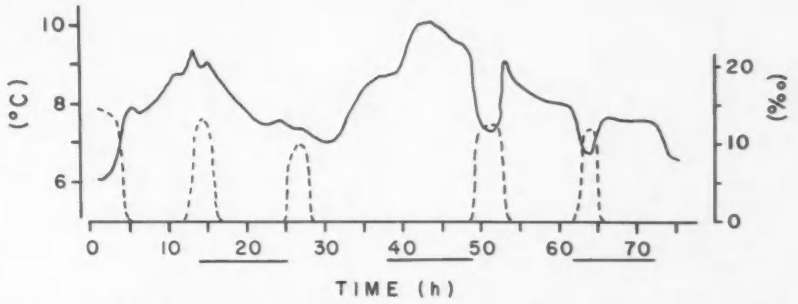

FIG. 4. Ouelle River water temperature (—) and salinity (---) recorded during the 72-h sampling series. Horizontal black bars indicate periods of darkness.

At the end of the series (hours 55–72), cool rains and persistent bad weather produced a period of continuously decreasing river water temperatures (Fig. 4).

May 1983 was marked by high rain fall in southeastern Quebec. The monthly mean of freshwater discharge in Ouelle River ($71.4\ m^3 \cdot s^{-1}$) exceeded the long-term (15 yr) mean by 30% ($54.4\ m^3 \cdot s^{-1}$, for May) (J. Deziel, Service de la météorologie, Division des expertises, Gouvernement du Québec, ministère de l'Environnement, 194 St-Sacrement, Québec, Qué. G1N 4J6, pers. comm.). The high runoff observed during the sampling period limited the upstream penetration of estuarine water during the flood in the river.

Smelt Larvae Abundance

Although the upstream spawning grounds were not localized in Ouelle River, exploratory sampling at the river's mouth detected the beginning of the hatching period. The 72-h larval sampling series was initiated at high slack water 2 d after the first yolk-sac larvae were caught in the river. The analysis of surface and bottom tows revealed great variability characterized by asynchronous peaks of larval abundance estimates at the surface and on the bottom (Fig. 5D). The surface sample series exhibits regularly spaced maxima at night associated with ebbing conditions in the river (Fig. 5D). These samples contained exclusively yolk-sac smelt larvae (mean length 6.04 ± 0.41 mm), suggesting that the 24-h fluctuations in larval abundance were most likely the result of discrete hatching events in the river.

Larval abundance maxima observed near the bottom, which also represent the highest larval densities recorded during the sampling period (Fig. 5D), are related to the high tides observed at the river station. These samples were composed of both yolk-sac and larger, post yolk-sac smelt larvae (mean length 6.52 ± 0.88 mm). The high densities of larvae in bottom tows and the presence of larger larvae observed at the river sampling station during the flood (Fig. 5C) suggest the aggregation of yolk-sac smelt larvae and their mixing with larval groups of estuarine origin nearby in the Baie de Ste-Anne area.

Vertical Distribution of Yolk-sac Smelt Larvae

Contingency table analyses performed to determine the effect of time of day and river current speed on the observed vertical position of larvae revealed that for riverine conditions (ebb periods, when there is no intrusion of salt or fluctuation in water level at the station), the vertical position of yolk-sac smelt larvae depends on light conditions (Table 1). In two cases, yolk-sac

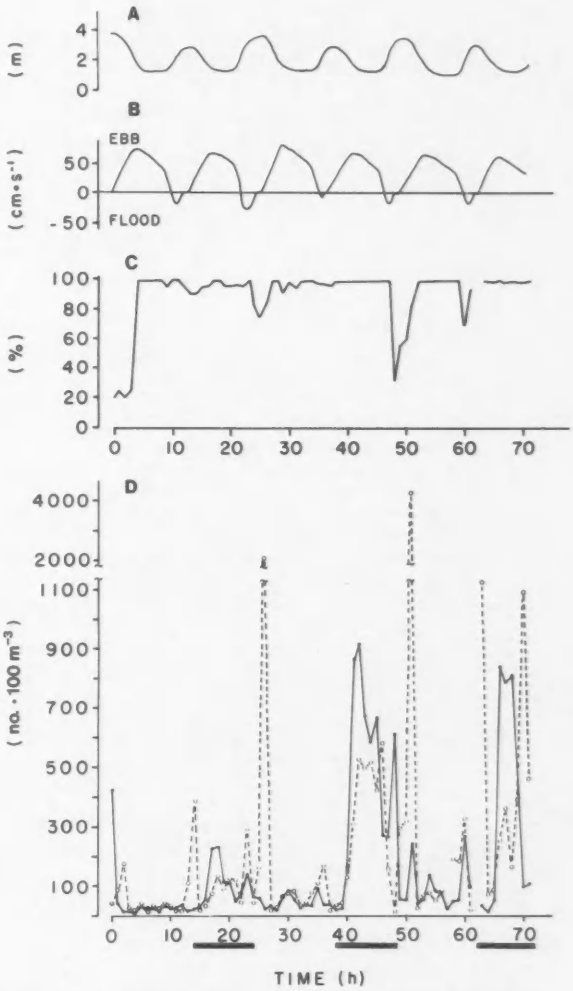

FIG. 5. Results of the 72-h sampling series (28–31 May 1983) in Ouelle River. (A) Tidal amplitude (m) at the river station; (B) tidal current velocity ($cm \cdot s^{-1}$) at the river station; (C) proportion (%) of yolk-sac larvae in the samples; (D) smelt larvae abundance estimates (no.·$100\ m^{-3}$); —, surface tows; ---, bottom tows. Horizontal black bars indicate periods of darkness.

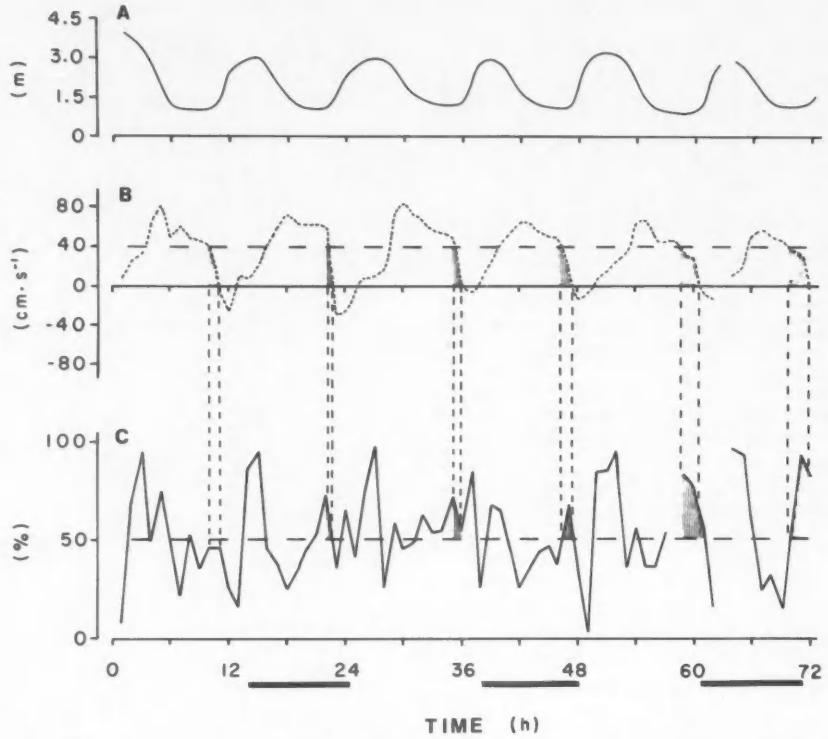

FIG. 6. Vertical position of smelt larvae and river hydrodynamics for the 72-h series. (A) Water level fluctuations (m); (B) current speed ($cm \cdot s^{-1}$) at the station (broken line indicates the 40 $cm \cdot s^{-1}$ critical speed); (C) proportion (%) of smelt larvae caught in bottom tows. Horizontal black bars indicate periods of darkness.

TABLE 1. Contingency table analysis. Abundance (no. · 100 m^{-3}) of yolk-sac smelt larvae sampled at each level during riverine conditions (ebb periods) for day and night conditions. The 72-h series was divided into three 24-h periods. $*p < 0.05$ a posteriori.

Sequence level	1		2		3	
	Day	Night	Day	Night	Day	Night
Surface	133	788	311	3967	407	2876*
Bottom	107	583	414*	2855	586*	2263
χ^2	0.132		62.1		75.0	
p	>0.5		<0.001		<0.001	

larvae were significantly more abundant near the bottom during daytime and, on one occasion, significantly more abundant near the surface at night (Table 1). To account for the phototaxic response of young smelt larvae, the contingency table analysis performed with river current speed was executed for day and night samples. Results revealed that during ebb phases of the tide, river current speed influences the vertical position of yolk-sac larvae in the water column during both day and night (Table 2). As river current speeds decrease, yolk-sac larvae appear to concentrate near the bottom. At flow velocities greater than 40 $cm \cdot s^{-1}$ the larvae are evenly distributed throughout the water column during the day, while they are more abundant near the surface at night (Table 2).

TABLE 2. Contingency table analysis. Abundance (no. · 100 m^{-3}) of yolk-sac smelt larvae sampled at different current speed intervals during ebb periods, by level and for day and night conditions. $*p < 0.05$ a posteriori.

Current speed ($cm \cdot s^{-1}$)	Day		Night	
	Surface	Bottom	Surface	Bottom
<30	91	109	209	581*
31–35	51	182*	0	0
36–40	78	230*	438	1495*
41–45	156	127	0	0
46–50	42	82	2053*	1098
51–55	26	28	788*	456
56–60	90	101	1617*	904
>61	295	248	2465*	1265
χ^2	132.8		1483.3	
p	<0.001		<0.001	

During the 72-h smelt larvae sampling period, the majority of larvae were observed in the bottom layer at high tides (Fig. 6). The percentage of larvae sampled in the bottom layer subsequently declined during the ebb. However, as current velocities decreased in the river near the end of the ebb, the proportion of larvae caught in the bottom layer once again increased, and at low slack water the majority of yolk-sac larvae were concentrated near the river bottom (Fig. 6).

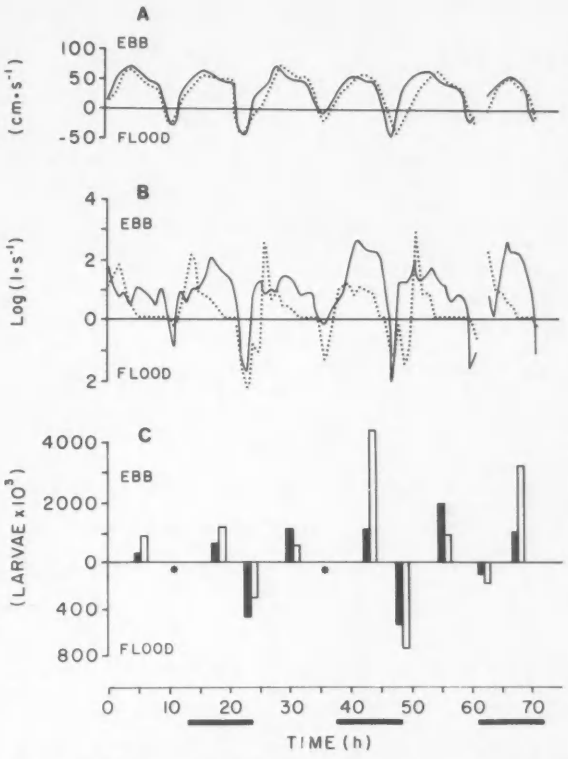

FIG. 7. Smelt larvae instantaneous flux and net exchange. (a) Current speeds in the surface (—) and bottom (···) layers of the river water column; (b) hourly larval flux estimates in the surface (—) and bottom (···) layers of the water column; (c) net transport estimates in the surface (open bars) and bottom (dark bars) samples for each phase of the six tidal cycles. Note change in scale for larval frequency during flood tides. Asterisks indicate $<100 \times 10^3$ larvae. Horizontal black bars indicate periods of darkness.

Larval Flux

Peaks of larval flux were observed in the bottom layer at the beginning of the ebb tide at the river station (Fig. 7A, 7B). High surface values of larval flux were continuously recorded during the nighttime ebb whereas surface values of flux are smaller and tend to decrease progressively during the daytime ebb (Fig. 7B). During the very short flood periods, high values of larval flux were recorded in both bottom and surface layers (Fig. 7B).

Estimates of net larval exchange per tidal cycle demonstrate a net exportation of smelt larvae into Baie de Ste-Anne (Fig. 7C). The transport of the most important numbers of larvae occurred in the surface layer during tidal cycles 2, 4, and 6 coincident with the important peaks of nighttime hatching (Fig. 5D). During tidal cycles 3 and 5, net exportation occurs principally in the bottom layer following the penetration of important larval concentrations of estuarine origin (Fig. 5D).

Discussion

Smelt Larvae Abundance Series

Data from the surface tows suggest the synchronous nighttime hatching of rainbow smelt larvae in Ouelle River. Although smelt larvae mean size observed in the low water samples (6.04 ± 0.41 mm) exceeds the size at hatching previously reported for smelt in Boyer River (mean length 5.51 ± 0.35 mm, Ouellet and Dodson 1985), the presence of yolk-sacs clearly indicates recent hatching in the vicinity of the sampling station. The differences in size may be due to the low water temperature experienced in May 1983 (Fig. 4). At low temperatures, the incubation time is longer and the larvae hatch at a more advanced developmental stage (Braum 1978). A similar 24-h periodicity in hatching related to light conditions was observed for the smelt larvae of Boyer River in 1982 (Ouellet and Dodson 1985), suggesting that the phenomenon may represent a characteristic of anadromous rainbow smelt life history in the St. Lawrence River.

River water temperatures cannot be considered as the factor controlling the 24-h periodicity in hatching of smelt larvae in Ouelle River. The progressive increase in water temperatures recorded over relatively broad periods of time in the series (Fig. 4) appears irrelevant to the occurrence of sharp larval abundance peaks observed during darkness. Moreover, the hatching peak recorded at the end of the series (hours 66–69, Fig. 5D) coincides with a period of decreasing and relatively low water temperatures. Also, the lack of a significant relationship between the abundances of yolk-sac smelt larvae and river discharge suggests that neither temperature nor river hydrodynamics control the nighttime hatching of larvae.

Alderdice and Velsen (1971) reported a diel cycle in hatching associated with light intensity for Pacific herring (*Clupea harengus pallasi*) larvae incubated in the laboratory. The majority of larvae hatched during darkness, but no mechanism was proposed to explain the phenomenon. No mechanism is immediately obvious for the control of the diel hatching cycle of smelt larvae, although the negative phototaxic response reported in earlier work for smelt larvae (Rogers 1940) and again documented recently in the St. Lawrence middle estuary (Ouellet and Dodson 1985) may be partially responsible. The activity of larvae may be enhanced by the reduction in light intensity prior to hatching, and such activity assists in breaking through the chorion (Blaxter 1969).

The occurrence of concentrations of smelt larvae during flood events at the sampling station and the presence of larger, post yolk-sac larvae in these high-water samples (Fig. 5C) indicates the mixing of yolk-sac larvae flushed from Ouelle River with groups of larvae retained in that zone since their drift from the river or with larval groups coming from other spawning sites in the estuary, possibly Boyer River. The estuarine origin of the larger smelt larvae is confirmed by the relation between tidal amplitude in the river and the importance of these larvae in the samples (Fig. 5A, 5C).

Vertical Position, Larval Flux, and Net Exchange of Yolk-sac Smelt Larvae

The contingency table analysis revealed that light conditions influence the vertical distribution of yolk-sac smelt larvae in the river (Table 1). However, the influence of river current velocities upon the vertical distribution of yolk-sac larvae, where at low current speeds smelt larvae aggregate near the bottom (Table 2), appears to be a more important element in the dynamics of the initial riverine drift. Newly hatched yolk-sac fish larvae have been described as poor swimmers and to experience passive sinking due to their high specific density while they are motionless (Blaxter and Ehrlich 1974; Weihs

1980; Kentouri and Divanach 1982). There is no reason for yolk-sac smelt larvae to be exceptional and we conclude that the yolk-sac larvae drifting in the river passively sink to the bottom as river flow decreases during both day and night conditions (Table 2).

The evolution of the vertical position of larval abundances shown in Fig. 6 illustrates that yolk-sac smelt larvae sank to the bottom prior to low slack water at the river's mouth preceding the intrusion of the estuarine water mass near the bottom during the flood. At this time, yolk-sac smelt larvae drifting from the river may be mixed with the estuarine water mass.

The instantaneous larval flux values calculated for each layer of the water column during the 72-h sampling series reflect the interaction between the vertical position of smelt larvae and river hydrodynamics. Flushing of intertidal water volumes from the river's mouth corresponds with large values of larval flux in the bottom layer of the water column (Fig. 7B). Larval flux in the bottom layer subsequently decreases rapidly and remains low once riverine conditions are reestablished. Larval flux peaks observed at the beginning of the ebb represent the flushing of high larval densities introduced during preceding floods (Fig. 7B) and indicate the back and forth advection across the sampling site of larvae already in the estuary or recently flushed from the river.

The flux of larvae in the surface layer presents a different pattern. For the duration of ebb tides in the river, larval flux values remain at a relatively constant high level, although larger values are observed at night (Fig. 7B). Nighttime synchronous hatching by smelt larvae is certainly the factor responsible for high larval flux in the surface layer at night. On these occasions, large numbers of yolk-sac smelt larvae are transported from the river to the estuary (Fig. 7C).

The vertical distribution and the flux of smelt larvae at the mouth of Ouelle River are reminiscent of the behavior of suspended sediment in this region. d'Anglejan et al. (1981) described the Baie de Ste-Anne as a temporary sediment trap and proposed that the phase difference between the ebb and flood tide in the river (Fig. 2) was the main factor responsible for retarding the seaward escape of riverine material producing the temporary accumulation of sediment observed in the area. High values of larval flux observed during the short flood events in the river and the mixing of yolk-sac smelt larvae emerged from Ouelle River with groups of larger larvae in the estuary suggest that smelt larvae are subject to similiar physical processes.

Although the seaward transport of smelt larvae is retarded resulting in temporary retention in the region of the mouth of Ouelle River, the longer ebb phases in the river are responsible for the net transport of smelt larvae towards Baie de Ste-Anne over the 72-h series (Fig. 7C). This pattern suggests that following the end of hatching in the river, smelt larvae would disappear from the vicinity of the natal river's mouth after only a few tidal cycles. Such a phenomenon was observed at the mouth of Boyer River in 1982 (Ouellet and Dodson 1985).

To summarize, although evidence was found of active phototaxic behavior of recently hatched yolk-sac smelt larvae, we reject the hypothesis that this behavior contributes to regulating the initial stages of larval drift. The riverine transport and mixing of yolk-sac smelt larvae with the estuarine water mass appears to be controlled principally by the hydrodynamics of the system and the nighttime hatching and specific gravity of yolk-sac larvae.

Acknowledgments

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Nutrient Availability for Phytoplankton Production in a Multiple-Impoundment Series[1]

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Elser, J. J., and B. L. Kimmel. 1985. Nutrient availability for phytoplankton production in a multiple-impoundment series. Can. J. Fish. Aquat. Sci. 42: 1359–1370.

We investigated the availability of nitrogen (N) and phosphorus (P) for phytoplankton production and the importance of inter-reservoir nutrient transfers in a headwater-to-mainstream series of three hypolimnetic-discharge impoundments during June–December 1982. Dissolved nutrient analyses and enrichment experiments indicated that P, rather than N, was the primary limiting nutrient for plankton growth throughout the reservoir series. Phytoplankton P deficiency, as reflected by chlorophyll-specific alkaline phosphatase activity (APA), peaked in late August, and then decreased during the fall and winter. Late in the growing season, APA levels increased downstream within each reservoir, reflecting uplake-to-downlake decreases in P availability, but decreased downstream from reservoir to reservoir, indicating increased P availability down the impoundment series. In this reservoir series, hypolimnetic releases of water and nutrients from upstream impoundments during the growing season appear to enhance nutrient availability for phytoplankton production in impoundments downstream. This result suggests that the productivity of reservoirs located in multiple-impoundment series should be viewed in the context of the limnological dynamics resulting from river basin-level reservoir operations.

De juin à décembre 1982, les auteurs ont étudié la disponibilité de l'azote (N) et du phosphore (P) pour la production phytoplanctonique, et l'importance du transfert de bioéléments entre bassins dans une série de trois réservoirs à débit hypolimnétique, des eaux d'amont au cours principal. Des analyses des bioéléments dissous et des expériences d'enrichissement ont révélé que P, et non N, était le principal bioélément limitatif de la croissance phytoplanctonique dans tous les réservoirs. La carence en P du phytoplancton, traduite par l'activité de la phosphatase alcaline (APA) particulière à la chlorophylle, a atteint une pointe à la fin d'août puis a diminué à l'automne et en hiver. Vers la fin de la saison de croissance, les niveaux d'APA ont augmenté dans les eaux d'aval de chaque réservoir, ce qui traduit des baisses de la disponibilité de P entre l'amont et l'aval, mais ils ont diminué en aval d'un réservoir à l'autre, ce qui révèle une disponibilité accrue de P de l'amont à l'aval de la série de réservoirs. Dans cette série, l'apport d'eau et de bioéléments provenant de la zone hypolimnétique des réservoirs d'amont pendant la saison de croissance semble accroître la disponibilité de bioéléments pour la production phytoplanctonique dans les réservoirs d'aval. Ce résultat porte à croire que la productivité de réservoirs en série devrait être envisagée dans le contexte de la dynamique limnologique résultant des opérations qui influent sur le niveau du bassin fluvial.

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Dam construction associated with water and power development projects has converted rivers throughout the world into regulated series of man-made impoundments. The effects of reservoir releases on downstream water temperature, flow regimes, water quality, and biota have received some attention (Young et al. 1972; Ward 1976; Ward and Stanford 1979, 1981); however, little is known of the ecological and limnological interactions occurring within series of impoundments. While natural lakes can also occur in series, hypolimnetic releases of water and nutrients from deep-discharge reservoirs may maximize the potential influence of upstream impoundments on the physical, chemical, and biological processes occurring in reservoirs downstream (Neel 1963; Wright 1967).

On an annual basis, impoundments are efficient sediment and phosphorus traps (Dendy et al. 1973; Heinemann et al. 1973; Gross et al. 1980, 1981). Except during the initial period of basin filling and nutrient leaching from inundated soils (Baxter 1977; Ostrofsky and Duthie 1980; Grimard and Jones 1982; Kimmel and Groeger 1985), the presence of a reservoir reduces the annual nutrient loading to downstream aquatic systems as a result of sedimentation losses of nutrients in the reservoir basin

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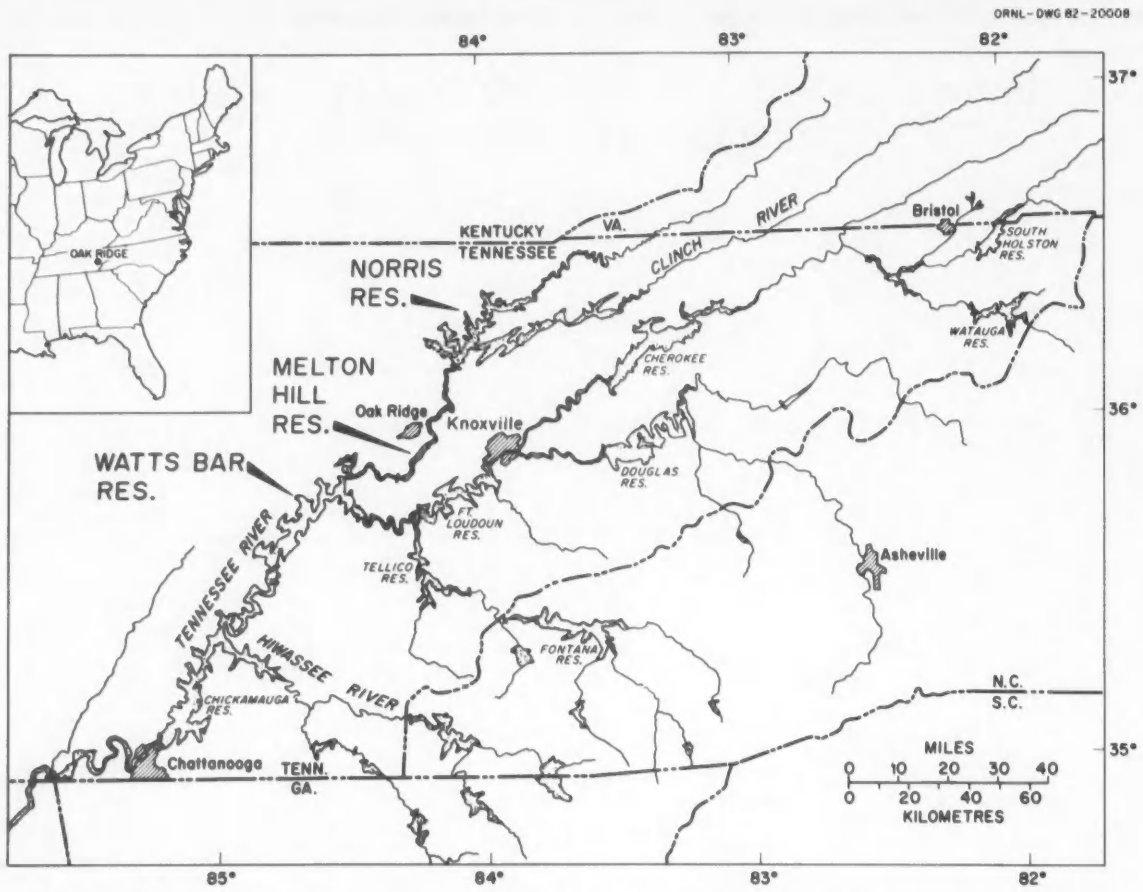

FIG. 1. Location of the Norris – Melton Hill – Watts Bar reservoir series in the upper Tennessee River valley, eastern Tennessee.

(Gloss et al. 1981; Paulson and Baker 1981; Daley et al. 1981). However, previous studies indicate that nutrient availability for phytoplankton production (versus total nutrient loading) may increase (Soltero et al. 1973; Martin and Arneson 1978; Priscu et al. 1982) or decrease (Gloss et al. 1980; Daley et al. 1981; Paulson and Baker 1981) in reservoirs downstream of deep-discharge impoundments. Numerous authors have emphasized the importance of outlet depth in regulating reservoir nutrient balance (Neel 1963; Wright 1967; Soltero and Wright 1975; Baxter 1977; Paulson and Baker 1981; Priscu et al. 1982).

Wright (1967) hypothesized that reservoirs with hypolimnetic discharges store heat and dissipate nutrients during the growing season as a result of releasing cold, nutrient-rich water, whereas surface-outflow reservoirs and natural lakes dissipate heat and trap nutrients by releasing warm, nutrient-poor water. Martin and Arneson's (1978) comparison of heat and nutrient distributions in a deep-discharge reservoir and in a surface-discharge lake supported Wright's hypothesis, but the in-lake biological impacts of the differing discharge regimes were difficult to discern. Wrights (1967) hypothesis suggests that, in series of deep-discharge impoundments, releases from upstream reservoirs should increase nutrient availability for phytoplankton growth in reservoirs downstream. If so, the effects of inter-reservoir nutrient transfers should be reflected in the spatial distribution of nutrient availability for phytoplankton growth within and among the reservoirs of a multiple-impoundment series.

The purpose of our investigation was to examine the longitudinal patterns of nutrient availability for phytoplankton production within a headwater-to-mainstream series of three reservoirs. We sought to address three questions: (1) Does nitrogen (N) and/or phosphorus (P) availability limit phytoplankton growth in the reservoir series? (2) What is the spatial and seasonal distribution of N and P availability within individual reservoirs? (3) Do the reservoir phytoplankton become P deficient in situ, and if so, does the degree of P deficiency vary within impoundments or along the headwater-to-mainstream reservoir gradient?

Materials and Methods

Description of Study Site

We studied three contiguous, Tennessee Valley Authority impoundments (Norris, Melton Hill, and Watts Bar reservoirs) comprising a 430-km headwater-to-mainstream reservoir series in eastern Tennessee (Fig. 1). Norris and Melton Hill reservoirs are tributary impoundments on the Clinch River. Watts Bar

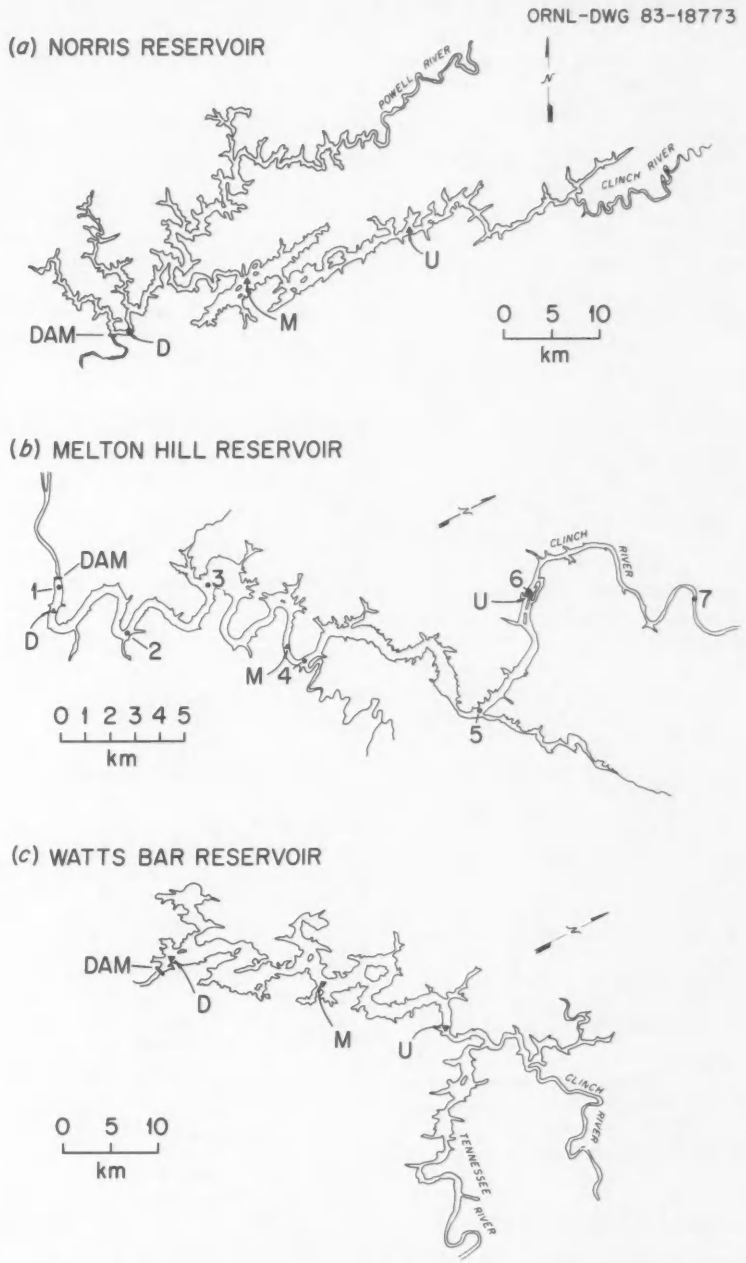

FIG. 2. Maps of (a) Norris, (b) Melton Hill, and (c) Watts Bar reservoirs, eastern Tennessee. Uplake (U), midlake (M), and downlake (D) sampling stations are indicated for each impoundment, and additional synoptic sampling stations (1–7) are shown for Melton Hill Reservoir.

Reservoir is a mainstream impoundment on the Tennessee River which receives Clinch River input from Melton Hill Reservoir. Norris (Fig. 2a), a headwater storage impoundment and the oldest of the three reservoirs, is relatively large and deep with complex basin morphometry (Table 1); it has a long residence time and is relatively unproductive (360 mg $C \cdot m^{-2} \cdot d^{-1}$ annual average, Taylor 1971). Melton Hill (Fig. 2b), the newest impoundment in the series, is relatively small, riverine, and has a short residence time. Watts Bar (Fig. 2c) has the largest surface area of the three impoundments, but also has a rather short residence time. All three reservoirs have hypolimnetic discharges and are used for flood control, water supply, power generation, and recreation. Melton Hill and Watts Bar reservoirs have locks permitting inter-reservoir navigation.

TABLE 1. Morphometric data for Norris, Melton Hill, and Watts Bar reservoirs, eastern Tennessee.

Parameter	Norris	Melton Hill	Watts Bar
Age (yr)	46	18	40
Drainage area, A_d (km^2)	7542	8658	44 833
Surface area, A_0 (km^2)	138	23	156
A_d:A_0 ratio	55	376	287
Length (km)	208	71	154
Shoreline development ratio	31	14	28
Mean depth (m)	18	6	8
Maximum depth (m)	61	21	21
Depth of outlet (m)	46	14	15
Mean annual water-level fluctuation (m)	18	3	2
Mean annual residence time (d)	237	15	18

Sample Collection and Field Measurements

The reservoir series was sampled on five occasions (June, July, August, October, December) during 1982. On each occasion, the three reservoirs were sampled on separate days within one week, at uplake, midlake, and downlake stations (Fig. 2). Water samples were obtained with a submersible pump from six depths within the mixed layer and the upper portion of the hypolimnion; sampling depths depended on relative light penetration and water depth at each station. At uplake and downlake stations, a 10-L water sample was obtained from 2-m depth for nutrient enrichment experiments and alkaline phosphatase assays. Samples were stored on-board in 10-L plastic containers, covered to prevent exposure to direct sunlight, and returned to the laboratory. The time elapsed between sampling and return to the laboratory depended on the lake and station being sampled, but did not exceed 4–5 h. Photosynthetically active solar radiation (PAR, 400–700 nm) and light attenuation were measured with a Li-Cor quantum meter with a submersible 4π sensor, and dissolved oxygen, pH, temperature,and conductance were determined using a Hydrolab 4041 monitoring system.

To better assess the spatial distribution of algal P deficiency within a reservoir, 2-m samples were collected at seven stations along the longitudinal axis of Melton Hill Reservoir (Fig. 2b) on August 23. Mixed-layer samples, for seston size fractionations, were also obtained from uplake, midlake, and downlake portions of Melton Hill on several dates. These samples together with the series sampling described above, produced a nearly biweekly sample record for Melton Hill Reservoir from July to December.

Dissolved Nutrients and Chlorophyll Analyses

Water samples for nutrient analyses were filtered through Whatman 934-AH glass-fiber filters, placed in acid-washed plastic bottles, and frozen until analysis. Soluble reactive phosphorus (SRP), NH_4-N, and $NO_2 + NO_3$-N concentrations were determined by standard techniques (Strickland and Parsons 1972; Stainton et al. 1974), using a Technicon II Autoanalyzer. The chlorophyll *a* concentration of each sample was measured in triplicate after filtration through glass-fiber filters (Whatman 934-AH), maceration with a tissue grinder, and methanol extraction (Marker et al. 1980). Filters were frozen until extractions were performed. For Watts Bar and Melton Hill samples, chlorophyll *a* was determined spectrophotometrically, with correction for phaeopigments (Lorenzen 1967). Due to very low chlorophyll levels, phaeopigment-corrected chlorophyll *a* concentrations of Norris Reservoir samples were determined fluorometrically (Holm-Hansen et al. 1965).

Nutrient Enrichment Experiments

Nutrient enrichment bioassays were performed on 2-m samples from uplake and downlake stations in each reservoir by allocating subsamples to eight 1-L clear, polyethylene bottles and making the following enrichments in duplicate: (1) +15 μg PO_4-P/L, (2) +15 μg NH_4-N/L, (3) +15 μg PO_4-P/L and +15 μg NH_4-N/L, and (4) control (no addition). Samples were then incubated in a growth chamber (15°C, 76 $\mu e \cdot m^{-2} \cdot s^{-1}$ PAR, 12:12-h photoperiod). Phytoplankton growth was monitored by measuring the chlorophyll in vivo fluorescence (IVF) of triplicate subsamples from each bottle every 24–48 h over a 4- to 5-d period. An Aminco fluorocolorimeter fitted with a blue excitation lamp (4 W/m^2 intensity, GE F4T5-B), a blue excitation filter (420 nm, CS5-60), a red emission filter (650 nm, CS2-64), and an R-136 photomultiplier was used to determine IVF levels.

Alkaline Phosphatase Assays

Alkaline phosphatase activity (APA) was assayed by the method of Pettersson (1980), using an Aminco fluorocolorimeter fitted with an ultraviolet lamp, a CS 7-60 primary filter (maximum transmission = 360 nm), and paired Wratten 8 and 48 secondary filters (maximum transmission = 450 nm). APA assays were performed in triplicate and results expressed as absolute APA (nmol PO_4-P·L^{-1}·min^{-1}) and as biomass-specific APA (nmol PO_4-P·μg chl a^{-1}·min^{-1}).

To determine the relative contribution of the phytoplankton to the observed APA levels, mixed-layer samples were obtained from Melton Hill Reservoir on 28 July, 6 September, and 1 October and size fractionated. Size fractionation was accomplished by gently vacuum-filtering (<100 mmHg (<13 kPa) pressure difference) small volumes (<50 mL) of sample through 1.0- and 0.2-μm pore size Nucleopore filters. The APA assay was conducted in triplicate on the two filtrates and on an unfiltered sample from each station, and the relative contributions of the three size fractions (<0.2, 0.2–1.0, >1.0 μm) were calculated by difference. To determine the efficiency of the size fractionation in separating algal from nonalgal particles, the chlorophyll IVF of fractionated and unfractionated samples was also determined.

Statistical Analyses

Results of nutrient enrichment bioassays were analyzed by analysis of variance (SAS Institute 1982). Scheffe's multiple comparison test (e.g. Snedecor and Cochran 1967) was applied to the 96-h IVF readings for each experiment, and differences among treatments were tested at the $\alpha = 0.01$ level. Fieller's Theorem for calculating sample variance for ratio data (Finney 1978) was employed to construct confidence limits for biomass-specific APA data, and differences among samples were tested at the $\alpha = 0.05$ level.

Results

Dissolved Nutrients

Concentrations of dissolved inorganic N and P indicated that, in the absence of other limiting factors, phytoplankton growth in

TABLE 2. Soluble reactive phosphorus (SRP), ammonia-N, and nitrite + nitrate-N concentrations (μg/L) and atomic ratios of dissolved inorganic nitrogen (DIN) and SRP for mixed-layer (2 m) samples from the Norris – Melton Hill – Watts Bar reservoir series, June–December 1982. DIN:SRP ratios calculated by summing NH_4-N and NO_2 + NO_3-N concentrations and setting SRP values <1 μg/L equal to 1 μg/L. U, M, and D represent uplake, midlake, and downlake sampling stations, respectively.

Date	Lake	Station	SRP	NH_4-N	$NO_2 + NO_3$-N	DIN:SRP
2–8 June	Norris	U	<1	10	60	>155
		M	—	—	—	—
		D	<1	10	200	>464
	Melton Hill	U	<1	<1	280	>621
		M	<1	20	5	>55
		D	<1	<1	3	>9
	Watts Bar	U	2	30	145	>192
		M	2	62	9	>77
		D	<1	<1	<1	>4
16–21 July	Norris	U	<1	9	1	>22
		M	<1	7	130	>82
		D	<1	8	270	>615
	Melton Hill	U	<1	<1	580	>1285
		M	<1	12	490	>1110
		D	<1	<1	290	>643
	Watts Bar	U	<1	<1	250	>555
		M	<1	30	2	>71
		D	<1	30	4	>75
23–27 August	Norris	U	<1	10	40	>111
		M	<1	<1	3	>9
		D	<1	<1	135	>301
	Melton Hill	U	<1	33	605	>1411
		M	<1	<1	470	>1042
		D	<1	26	420	>986
	Watts Bar	U	<1	10	300	>686
		M	<1	29	115	>318
		D	<1	12	73	>188
4–8 October	Norris	U	<1	<1	22	>51
		M	<1	<1	40	>91
		D	<1	<1	220	>489
	Melton Hill	U	2	80	320	>885
		M	<1	22	390	>911
		D	<1	<1	415	>920
	Watts Bar	U	<1	8	433	>975
		M	<1	30	400	>951
		D	<1	<1	260	>577
7–13 December	Norris	U	2	60	728	>871
		M	<1	20	225	>542
		D	<1	16	235	>555
	Melton Hill	U	3	30	310	250
		M	2	18	400	462
		D	13	28	440	80
	Watts Bar	U	23	99	415	49
		M	15	60	425	71
		D	13	54	334	66

the reservoir series was likely to be P limited. SRP levels in the reservoir series were usually below the limit of analytical detection (<1.0 μg/L) during the growing season both in samples from the mixed layer (Table 2) and from the upper portion of the hypolimnion (Elser 1983). Detectable SRP was present in most samples taken in December, with the lowest levels (<3.0 μg/L) occurring in Norris and the highest in Watts Bar (11.0–24.0 μg/L). Throughout the study, nitrate-N levels were higher than SRP and ranged up to 728 μg/L in December. Ammonia-N was lower than NO_2 + NO_3-N, but occasionally reached concentrations of 50–100 μg/L (Table 2). Atomic ratios of dissolved inorganic nitrogen (DIN) and SRP were

TABLE 3. Average (all stations) dissolved inorganic nitrogen levels (NH_4-N + NO_2 + NO_3-N in µg/L) for mixed-layer (2 m) samples from Norris, Melton Hill, and Watts Bar reservoirs, June–December 1982.

	Jule	July	August	October	December
Norris	260	129	64	29	427
Melton Hill	109	437	498	417	407
Watts Bar	44	111	213	321	363

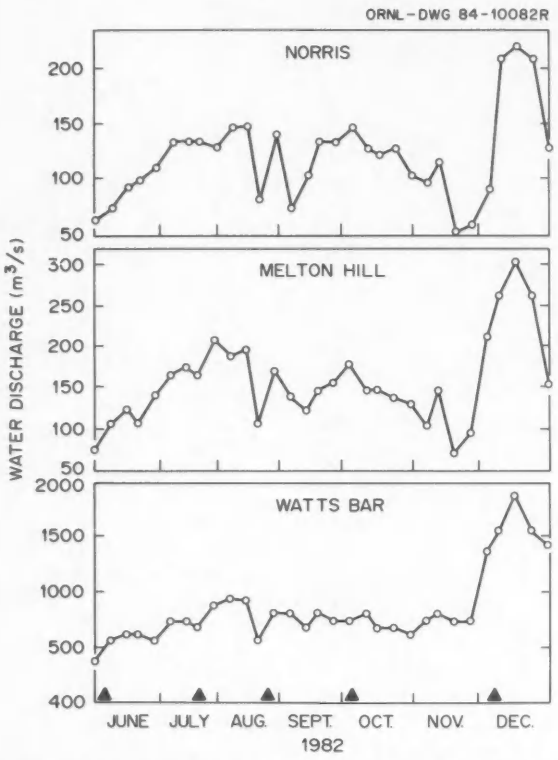

FIG. 3. Rates of water discharge (weekly averages) from Norris, Melton Hill, and Watts Bar dams during June–December 1982. Note changes of scale for discharge rates among reservoirs. Solid triangles along the abscissa represent sampling periods.

generally >30:1; occasional lower DIN:SRP ratios resulted from decreased DIN concentrations rather than from increased SRP (Table 2).

Marked changes in mixed-layer DIN occurred in the reservoir series during the June–December study period (Table 3). In June, DIN concentrations decreased along the headwater-to-mainstream reservoir series from Norris to Melton Hill to Watts Bar. However, concurrent with increases in reservoir hypolimnetic discharges associated with increased peaking power generation (Fig. 3), DIN concentrations increased in Melton Hill between our June and July sampling dates. During July–October, mixed-layer DIN levels in Norris declined by 50% at each sampling date and did not increase again until fall overturn (between our October and December samples). DIN concentrations increased steadily in Watts Bar throughout the study period.

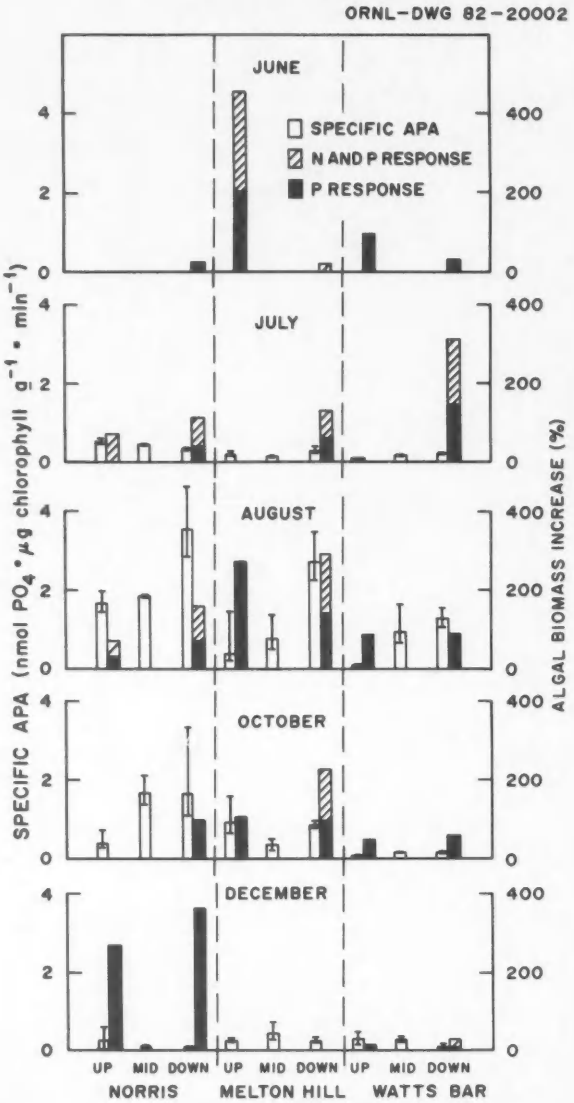

FIG. 4. Within- and among-reservoir patterns of chlorophyll-specific alkaline phosphatase activity (APA) and algal growth responses to nutrient enrichment for mixed-layer (2 m) samples from the Norris – Melton Hill – Watts Bar series, June–December 1982. Four-day bioassay responses to P enrichment and to combined N and P enrichment are expressed as the percent increase in algal biomass (as reflected by chlorophyll in vivo fluorescence) over unenriched controls; only statistically significant ($P < 0.01$) biomass increases are shown. Error bars represent 95% confidence limits for APA values.

Nutrient Enrichment Experiments

Nutrient enrichment results also indicated potential P limitation of in situ phytoplankton productivity. Significant ($P < 0.01$) stimulation of phytoplankton growth in P-enriched samples occurred in 22 of 30 (73%) nutrient enrichment experiments (Fig. 4), although the degree of the reponse to enrichment was variable. Significant responses to N enrichment occurred on

only three occasions: uplake in Norris in July and uplake in Watts Bar in August and December (Elser 1983). Enhanced responses to a combined N + P treatment (i.e. biomass levels significantly ($P < 0.01$) higher than those with a N or P addition alone) occurred in 9 of the 30 experiments, most commonly in July and August samples (Fig. 4). Uplake samples usually responded less to P and N + P enrichments than did downlake samples (Fig. 4). Among-reservoir patterns were less apparent, but, in general, enrichment bioassay results indicated that potential P limitation of phytoplankton growth was common to all three reservoirs.

Alkaline Phosphatase Assays

APA size fractionation

Chlorophyll IVF measurements on size-fractionated plankton samples showed that 90% of the algal chlorophyll occurred in the >1.0-μm particle size fraction (Elser 1983). Size fractionation of APA in Melton Hill Reservoir samples on 28 July, 6 September, and 1 October revealed that, with the exception of the uplake station on 28 July (discussed below), phytoplankton-size particles (>1.0 μm) accounted for 49–90% ($\bar{x}$ = 74%) of the total APA. Dissolved (<0.2 μm) APA ranged from 0.4 to 26% ($\bar{x}$ = 12%) of the total (Table 4). Similarly, size fractionation of APA in mixed-layer samples from the three Norris stations on 8 October indicated that only 10–15% of the total APA was in the dissolved fraction (Elser 1983). An exception to this pattern occurred on 28 July at the uplake station in Melton Hill Reservoir where only 7% of the total APA was associated with >1.0-μm particles and 50% was in the <0.2-μm fraction. Effects of the Norris Reservoir hypolimnetic release where likely responsible for this observation. At this time, the Melton Hill uplake station was characterized by cold, clear, low-chlorophyll water discharged from the Norris hypolimnion. Because little algal biomass (<0.05 μg chl *a*/L) was present, the nonalgal fraction (dissolved and bacterial APA, as indicated by APA in the <0.2- and 0.2- to 1.0-μm size fractions, respectively) dominated the total sample APA, which was low (0.09 nmol PO_4-P·L^{-1}·min^{-1}).

Our size-fractionation results indicated that dissolved and bacterial APA usually contributed to total APA values to a minor extent (Table 4). Therefore, we attributed the observed APA levels to phytoplankton, and interpreted APA patterns in terms of in situ phytoplankton P status.

Temporal and spatial patterns in APA

APA levels generally decreased and algal chlorophyll increased downstream along the headwater to mainstream reservoir gradient (except in December following fall overturn, Fig. 5). Significant ($P < 0.05$) differences in biomass-specific APA levels occurred between mixed-layer and hypolimnetic samples in Norris and Melton Hill reservoirs in August (Fig. 5) although SRP concentrations were consistently <1 μg/L in both epilimnion and upper hypolimnion samples (Elser 1983). Because significant differences in APA did not occur among mixed-layer samples (Fig. 5), we concluded that APA levels of 2-m samples were representative of mixed-layer phytoplankton at each station.

In situ phytoplankton P deficiency, as reflected by specific APA levels, increased during the summer, peaked in August, and then decreased during the fall and winter (Fig. 4). Longitudinal gradients in APA within impoundments were most pronounced late in the growing season (August and October). In each of the three reservoirs, downlake APA was usually higher than that at uplake and midlake stations.

TABLE 4. Size fractionation of alkaline phosphatase activity (APA) in mixed-layer samples from Melton Hill Reservoir. Data expressed as the percentage of the total sample APA occurring in each size fraction. U, M, and D refer to uplake, midlake, and downlake stations, respectively.

Size fraction (μm)	28 July			6 September			1 October
	U	M	D	U	M	D	D
>1.0	7.2	89.7	79.7	70.4	77.2	48.9	77.9
0.2–1.0	42.8	9.8	8.6	14.8	13.0	24.8	15.6
<0.2	50.0	0.4	11.6	14.8	9.8	26.3	6.5

Synoptic Melton Hill data (Fig. 6) for 23 August most clearly reflect the occurrence of longitudinal gradients in specific APA levels, as well as in other variables, within a reservoir. Chlorophyll *a* concentrations increased steadily downlake. SRP concentrations were <3.0 μg/L throughout the longitudinal transect, but were highest at station 7 in the Clinch River upstream from Melton Hill Reservoir and at station 1 near Melton Hill Dam (Fig. 2 and 6). The discharge of nutrients from the Norris hypolimnion explains higher SRP concentrations at station 7, and we believe that upwelling of deeper, more nutrient-rich water occurs at station 1 as a result of peaking power generation–discharge cycles. We commonly observe elevated dissolved nutrient concentrations and phytoplankton productivity at near-dam stations in hydropower reservoirs (B. L. Kimmel, unpubl. data).

Longitudinal patterns for absolute and specific APA were parallel, except for the uppermost station (station 7) where low algal biomass resulted in an anomalously high biomass-specific APA value, as discussed above for the uplake station on 28 July. Specific APA levels at the three most downlake Melton Hill stations (stations 1, 2, and 3) were not statistically different from each other, but were significantly higher ($P < 0.05$) than those at stations 4, 5, and 6. Station 4 APA was significantly higher than that further uplake at stations 5 and 6 (Fig. 6). These data demonstrate a marked, within-reservoir transition from a condition of low algal P deficiency at uplake, riverine stations (stations 5 and 6), to an intermediate condition at midlake (station 4), to high algal P deficiency at downlake, lacustrine stations (stations 1–3).

Melton Hill Reservoir data also provide a detailed view of the temporal pattern of algal biomass and specific APA levels within a single impoundment (Fig. 7). Chlorophyll levels were consistently low at the uplake station, and higher, but more variable, at midlake and downlake stations. Specific APA increased ($P < 0.05$) at midlake and downlake stations between 28 July and 23 August, and then declined; among-station differences were greatest during peak APA levels in August. Statistically significant ($P < 0.05$) differences among stations only occurred late in the growing season (late August – early October) (Fig. 7). Specific APA levels at the Melton Hill uplake station exceeded ($P < 0.05$) those at midlake in September and October (Fig. 7), again, probably a result of the low chlorophyll content of the Norris discharge.

During most of the growing season (July–October), the magnitude of biomass-specific APA levels decreased from reservoir to reservoir moving down the Norris – Melton Hill – Watts Bar series (Fig. 4). Highest levels of specific APA

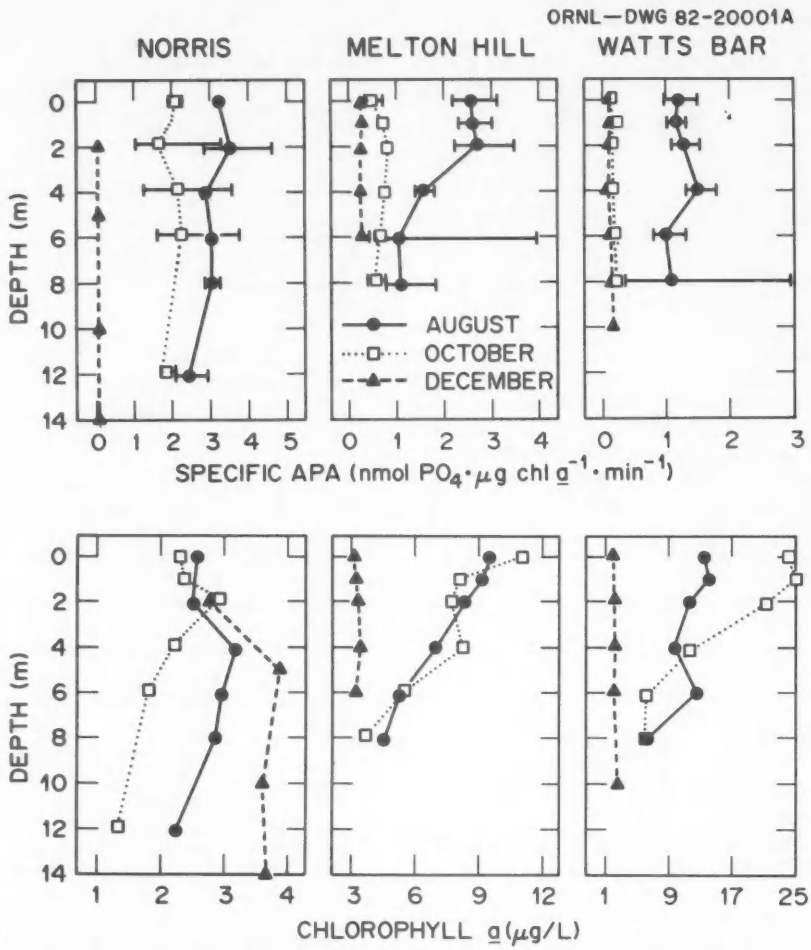

FIG. 5. Vertical distributions of chlorophyll-specific alkaline phosphatase activity (APA) and chlorophyll *a* concentrations at downlake stations in Norris, Melton Hill, and Watts Bar reservoirs, August–December 1982. Error bars indicate 95% confidence limits for APA values; where error bars are not visible, the confidence limit range is less than the width of the symbol. Note changes of scale among reservoirs.

occurred in late August, decreased again by early October, and then declined to very low levels in December. Specific APA levels in all three reservoirs in December were similar in magnitude to those at uplake stations at other times of the year.

Discussion

Concentrations of SRP were consistently low and frequently undetectable in the Norris – Melton Hill – Watts Bar reservoir series during the growing season. Undetectable levels of SRP do not demonstrate that P is unavailable for phytoplankton uptake, however. Phosphorus pools are highly dynamic in freshwater ecosystems and PO_4-P is regenerated rapidly (Lean 1973; Francko and Heath 1979; Nalewajko and Lean 1980; White et al. 1981). Additionally, it is unclear whether phytoplankton or bacteria are responsible for most PO_4-P uptake in nutrient-poor aquatic systems. Recent results suggest that the bacterioplankton may dominate PO_4-P uptake and cycling and the phytoplankton may obtain some of its P from excreted organic P compounds (Currie and Kalff 1984a, 1984b; Ammerman and Azam 1985). We did not compare PO_4-P uptake by algae and bacteria; however, our chlorophyll and APA size-fractionation measurements indicated that most of the APA was usually associated with phytoplankton rather than with bacteria in these reservoirs.

The ratio of inorganic N to inorganic P in solution provides an informative, preliminary indicator of nutrient availability for phytoplankton production (Vincent 1981a, 1981b). Throughout the reservoir series examined in this study, low SRP concentrations were accompanied by relatively high inorganic N concentrations. DIN:SRP atomic ratios generally exceeded 30:1, the value usually considered to indicate P limitation in algae (Rhee 1978; Goldman and Peevey 1979), and were well above the cellular composition ratio of 17:1 for phytoplankton growing at a maximum rate (Redfield 1958; Redfield et al. 1963). Therefore, our dissolved nutrient analyses indicated that P, rather than

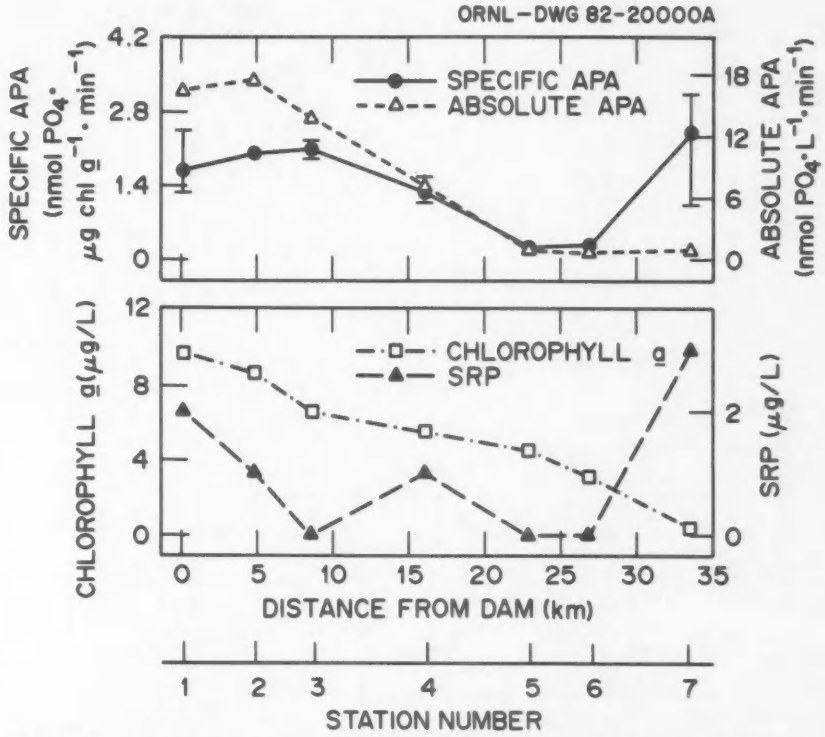

FIG. 6. Levels of absolute and biomass-specific alkaline phosphatase activity (APA), and concentrations of chlorophyll *a* and soluble reactive phosphorus (SRP) in mixed-layer (2 m) samples collected along the longitudinal axis of Melton Hill Reservoir, 23 August 1982. Error bars indicate 95% confidence limits for APA values, as in Fig. 5.

N, would be the primary limiting nutrient for phytoplankton growth in these reservoirs. However, due to low SRP concentrations and the dominance of DIN:SRP ratios by $NO_3 + NO_2$-N, within-reservoir gradients in P availability were not directly evident from the chemical analyses.

The results of nutrient enrichment experiments also indicated P limitation of phytoplankton production in the reservoir series. Enrichment experiments can only indicate the potential for nutrient limitation of in situ phytoplankton production in the absence of other limiting factors (e.g. see Healey and Hendzel 1980; Vincent 1981a, 1981b). However, on a relative basis, algal growth responses to experimental P and N + P additions were usually less in uplake samples than in downlake samples, indicating an uplake-to-downlake decline in P availability within reservoirs.

The degree of in situ algal P deficiency, as indicated by chlorophyll-specific APA levels, varied temporally and spatially in the reservoir series. An inverse relationship between APA and internally stored P in algae is well known (Fitzgerald and Nelson 1966; Healey and Hendzel 1980; Petterson 1980; Vincent 1981a; Wynne 1981), and the rapid increase in biomass-specific APA observed between July and late August probably reflected phytoplankton assemblages becoming P deficient as internal P stores were exhausted. Spatially, APA levels decreased from reservoir to reservoir during most of the growing season, indicating that P availability increased downstream in the impoundment series. Longitudinal gradients in APA in each reservoir were most pronounced in late August, indicating uplake-to-downlake increases in phytoplankton P deficiency and, presumably, corresponding to downlake decreases in P availability. Similar longitudinal patterns in APA and P availability have been observed in Lake Memphremagog (Quebec–Vermont), an elongate glacial lake having an uplake-to-downlake nutrient gradient resulting from river inflow (Sproule and Kalff 1978; Peters 1979; Smith and Kalff 1981), and in Southern Indian Lake (Manitoba) prior to its lake level elevation by impoundment (Healey and Hendzel 1980; Hecky and Guildford 1984).

Limiting factors other than P availability, which were likely responsible for discrepancies between results of nutrient enrichment experiments and APA assays (Elser 1983), could affect the spatial distribution of algal P deficiency. In particular, uplake stations in all three impoundments are riverine and are characterized by deeper vertical mixing and more rapid flushing than occur at downlake sites. At times, in situ phytoplankton production at these uplake stations may be more limited by light or flushing rate than by P availability. Consistently high DIN:SRP ratios maintain a potential for the development of P limitation and, thereby, cause uplake samples to exhibit both low APA levels and positive algal growth responses to experimental P enrichment. Although the influence of other limiting factors at uplake stations could affect the apparent within-reservoir spatial distribution of algal P deficiency as indicated by APA levels, the results of nutrient enrichment experiments are independent of nonnutritional limitations and support the pattern of an uplake-to-downlake decline in P availability.

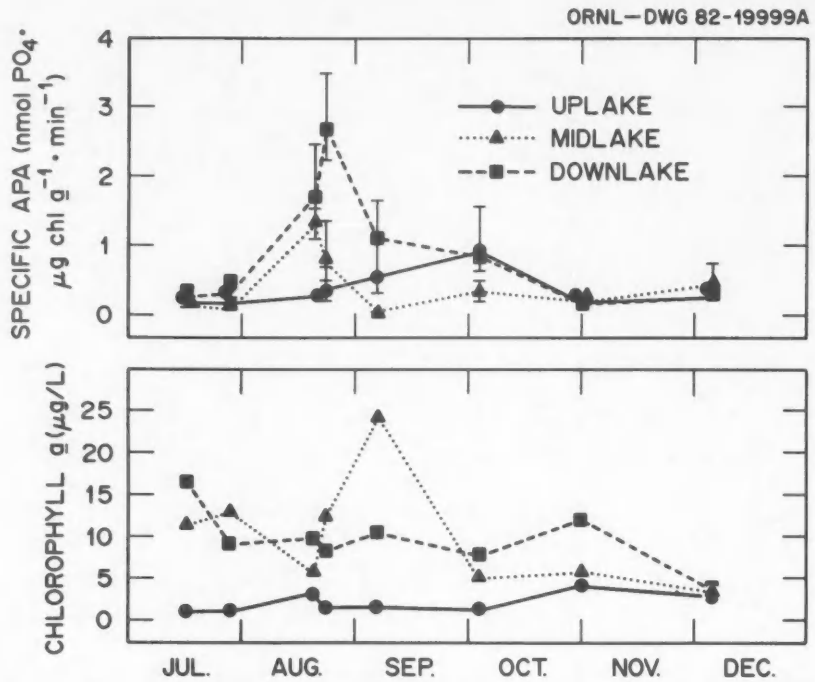

FIG. 7. Seasonal patterns of biomass-specific alkaline phosphatase activity (APA) and chlorophyll *a* concentrations in mixed-layer (2 m) samples from uplake, midlake, and downlake stations in Melton Hill Reservoir, July–December 1982. Error bars indicate 95% confidence limits for APA values, as in Fig. 5 and 6.

Plant nutrients, particularly P, are efficiently retained in storage reservoirs (Dendy et al. 1973; Heinemann et al. 1973; Gloss et al. 1980, 1981); for example, the annual trapping efficiency of Norris Reservoir is 60% for total P and 34% for total N (Iwanski et al. 1980; Placke 1983). However, the combined results of DIN and SRP analyses, nutrient enrichment experiments, and APA assays indicate that hypolimnetic discharges from Norris increased nutrient availability in downstream reservoirs during the growing season. The sharp increase in DIN in Melton Hill between our June and July sampling dates corresponded to increased Norris releases associated with peaking power generation. That the DIN increases in Melton Hill and Watts Bar reservoirs resulted from hypolimnetic discharges upstream is indicated by the steady decline of mixed-layer DIN concentrations in Norris Reservoir, which receives no inputs from upstream deep-discharge impoundments. Thus, changes in mixed-layer DIN concentrations in the three reservoirs during the study period show that hypolimnetic releases substantially affected the transport and distribution of DIN in the reservoir series and suggest that P distribution and its availability for phytoplankton production was similarly influenced.

Both uplake-to-downlake increases in chlorophyll-specific APA levels and the results of nutrient enrichment experiments indicated that, relative to the releasing reservoir, P availability increased in the uplake portion of reservoirs receiving hypolimnetic discharges from upstream. Although higher SRP concentrations were generally not detected at routine uplake sampling stations, increased SRP levels at the synoptic sampling station (station 7) located in the Clinch River between Norris and Melton Hill reservoirs provided evidence of SRP transfer from Norris to Melton Hill. The reservoir-to-reservoir decrease in APA through the Norris – Melton Hill – Watts Bar impoundment series late in the growing season (August–October) also supports the hypothesis that hypolimnetic discharges from upstream reservoirs increase nutrient availability in downstream impoundments.

Other factors may also contribute to the observed distribution of nutrient availability and phytoplankton P deficiency in the reservoir series, however. Multiple-impoundment series generally encompass a broad range of environmental conditions, and it is difficult to separate the effects of hypolimnetic nutrient releases from effects of drainage basin size, reservoir hydrodynamics, basin morphology, and flushing rates. For example, downstream reservoirs tend to drain a larger cumulative area. In this reservoir series, Norris and Melton Hill drain approximately the same watershed, but Watts Bar also receives drainage from the upper reaches of the Tennessee River basin which includes the city of Knoxville (Fig. 1). Therefore, lower P deficiency in Watts Bar likely also reflects a higher P loading rate. Additionally, in relatively shallow reservoirs such as Melton Hill and Watts Bar, riverine inflow passing through the reservoirs as subsurface flows may interact with the trophogenic zone to a greater extent than it would in deeper basins, such as Norris, and thereby increase the effective nutrient supply to the euphotic layer. Furthermore, higher flushing rates probably increase rates of advective nutrient supply in upper portions of Melton Hill and Watts Bar relative to that in most of Norris Reservoir and, thereby, may be partially responsible for decreased algal P deficiency in the downstream reservoirs. Given

these complications, however, our results, in particular (1) the temporal redistribution of DIN (and, presumably, SRP) in the reservoir series, (2) the uplake-to-downlake decreases in P availability within reservoirs as indicated by nutrient enrichment experiments and APA assays, and (3) the reservoir-to-reservoir increases in P availability down the reservoir series, suggest the ecological importance of nutrient transfers from upstream impoundments to reservoirs downstream.

In the absence of an upstream impoundment, nutrient inputs to reservoirs are usually seasonal and associated primarily with periods of high precipitation or snowmelt in winter and spring (Daley et al. 1981; Ward and Stanford 1983; Kimmel et al. 1985). Because of suboptimal growth conditions (low light, low water temperature) and high flushing rates, nutrient inputs at these times of maximal inflow may not be utilized as efficiently by reservoir phytoplankton as nutrient inputs occurring later in the growing season. Retention of spring runoff in storage reservoirs and the subsequent gradual release of water and nutrients as a consequence of hydropower operations extend the period of significant downstream nutrient transport through the growing season. We hypothesize that this temporal redistribution of the nutrient supply to downstream reservoirs results in a more efficient conversion of the annual nutrient supply to phytoplankton production than would occur in the absence of upstream, deep-discharge impoundments. This hypothesis presumes that a significant portion of the nutrients released from an upstream impoundment during the growing season enters the euphotic zone of the reservoir downstream (e.g. via mechanisms such as those described by Carmack and Gray 1982 and Fischer and Smith 1983) rather than being confined to aphotic layers. Density flows are often enhanced by hypolimnetic releases, however, and the effective nutrient supply to the trophogenic zone of a thermally stratified, downstream reservoir may be significantly less than the upstream nutrient discharge (Paulson and Baker 1981; Kimmel et al. 1985). An improved understanding of the extent to which nutrients released in hypolimnetic discharges enter or are isolated from the euphotic layer in impoundments downstream will be required to determine more specifically the influence of deep-discharge releases on the biological productivity of multiple-impoundment series.

Series of large, multiple-purpose reservoirs (e.g. those located on the Columbia, Colorado, Missouri, Arkansas, and Tennessee rivers) are operated on an integrated, river-basin scale to provide flood control, hydroelectric power, water supply, and, in some cases, navigation. Our results and those of previous investigators (Martin and Arneson 1978; Gloss et al. 1981; Paulson and Baker 1981; Daley et al. 1981; Priscu et al. 1982) clearly indicate that the ecology of reservoirs located in multiple-impoundment series must be viewed in the context of the limnological dynamics resulting from basin-level reservoir operations. Our data also suggest that, during the growing season, reservoir hypolimnetic releases associated with hydropower operations influence the supply of nutrients available for phytoplankton production in reservoir series, a result consistent with the implications of Wright's (1967) hypothesis regarding the downstream dissipation of nutrients by deep-discharge reservoirs.

It will remain difficult to specifically evaluate the importance of inter-reservoir nutrient transfers because other factors (e.g. differences in drainage basin size, reservoir basin morphology, hydrodynamics, intermediate nutrient sources, and limiting factors other than nutrients) also influence nutrient availability and phytoplankton productivity in reservoir series. However, because of the potential value of integrating multiple-impoundment operation and scheduling practices with reservoir fishery production and water quality management objectives, further research to determine the influence of inter-reservoir nutrient transfers on biological productivity in multiple-impoundment series is warranted.

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A Stochastic Population Model for Managing the Atlantic Menhaden (*Brevoortia tyrannus*) Fishery and Assessing Managerial Risks

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A model including an age-structure, a stochastic egg-recruitment relationship, density-dependent juvenile growth, age-dependent fishing mortality, and fecundity dependent upon size as well as age was used to investigate three types of harvesting strategies: constant yearly catch policies, constant fishing mortality rate policies, and "egg escapement" policies, which are defined in the article. Because of stochastic recruitment, constant yearly catch policies appear unsuitable for managing Atlantic menhaden (*Brevoortia tyrannus*). Both other types of policies are suitable, but the egg escapement policies have higher long-term average catches. Using decision theory, we investigated risks due to the randomness of recruitment and to the estimation errors for the biographical parameters in our model. The risks appear to be acceptable.

Un modèle structuré selon l'âge englobant une relation stochastique entre le recrutement et le nombre d'œufs, une croissance des juvéniles liée à la densité, une mortalité par pêche liée à l'âge et une fécondité liée à la taille et à l'âge a servi à l'étude de trois stratégies d'exploitation : un régime de prises annuelles constantes, un régime visant un taux constant de mortalité par pêche et un régime axé sur « l'échappée d'œufs » défini dans la présente communication. À cause du recrutement stochastique, le premier régime ne semble pas convenir à la gestion de l'alose tyran (*Brevoortia tyrannus*). Les deux autres sont appropriés quoique le dernier permette des prises moyennes à long terme plus élevées. À l'aide de critères de décision, les auteurs ont examiné les risques inhérents au caractère aléatoire du recrutement et aux erreurs d'estimation relatives aux paramètres biographiques du modèle, risques qui semblent acceptables.

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The Atlantic (*Brevoortia tyrannus*) and Gulf (*Brevoortia patronus*) menhaden together support the largest United States commercial fishery in biomass, and, excluding shellfish, menhaden catches rank next to those of salmon and tuna in monetary value. Catches of Atlantic menhaden rapidly declined after 1962; a total catch of 537 000 metric tons (t) in 1962 dropped to 193 000 t in 1967 (Nicholson 1971). Recent catch data show that the Atlantic menhaden fishery has only partially recovered. We developed a simulation model of the Atlantic menhaden fishery to investigate various harvesting policies and their associated risks.

Beddington et al. (1981) have noted that there are two distinct roles for mathematical models of renewable resources: (1) understanding and suggestion of general biological and economic principles and (2) management. Models of the first type are often simple and analytically tractable, while models in the second category contain detailed structures which generally improve their predictive performance.

We used a detailed management-oriented model. The model was structured to reflect information from a large data base collected from 1955 through 1979 by the National Marine Fisheries Service Laboratory at Beaufort, NC, and many suggestions from the staff there. An extensive analysis of these data is found in Reish et al. (1985). Important features of menhaden biology were incorporated into the model, including an age-structure, density-dependent juvenile growth, age-dependent

fishing mortality, size, and age-dependent fecundity, and a stochastic egg-recruitment relationship.

We investigated three classes of management policies: (1) constant yearly catch policies, (2) constant instantaneous fishing mortality (F) policies, where the age-specific F's are kept fixed, and (3) policies we call "egg escapement" policies where the egg production by the mature stock is controlled, though not completely. We chose to investigate these policies primarily because they are all stationary policies (Reed 1974) where harvest in a given year depends only on some index of abundance and not on the year of harvest. (An exception is the "phase-in" time-dependent policy presented in connection with policy (1).) Secondly, they span the three classes of controls available to a fishery manager: in (1), catch is controlled; in (2), effective fishing effort is controlled; and in (3), escapement for spawning is controlled. Each of these policies has advantages and disadvantages which we discuss individually in this article. Most of these policies have been examined in various stochastic autonomous population models (e.g. Evans 1981; Ludwig 1980; Hilborn 1979; Hutchinson and Fischer 1979; May et al. 1978; Mendelssohn 1982; Reed 1974, 1978; Walters and Hilborn 1976). By an autonomous model we mean a model where the differential or different equations of population change are a function of a single state variable, e.g. biomass of total stock, rather than, say, of age-specific biomasses. The term "autonomous" is borrowed from the theory of differential equations. Nonautonomous age-structured stochastic models have received much less attention, although a general theory for age-specific harvesting is available (Mendelssohn 1978). We believe that ours is the first study of a particular fish stock where these three stationary policies are compared in an age-structured stochastic population model.

To analyze these policies and their associated risks, we used decision theory, the science of making decisions under conditions of uncertainty. Fisheries management entails uncertainty because the model parameters are estimated with error and because the actual fish populations usually exhibit random variations. Even if our model were exact and all parameters were known, we might still face considerable uncertainty if the population had stochastic components. (Another cause of risk, uninvestigated here, is model misspecification.)

From decision theory, we borrowed the ideas of "utility function" and "opportunity loss." Although we did not make extensive use of formal decision theoretic methods, we found these tools very useful when measuring risk.

The Biological Model

The model predicts yearly catches of menhaden on the Atlantic coast. More complex (area- and season-specific) models than ours did not seem feasible, partly due to time and cost constraints, but largely because of inadequate data. Although a large amount of catch-effort and mark–recapture data are available, they do not provide accurate estimates of migration rates and area- and season-specific natural mortality rates (Reish et al. 1985).

The egg-recruitment data were well fitted by the Beverton-Holt model (Beverton and Holt 1957):

$$(1)\quad N_0 = 1/(\alpha + \beta/E)$$

where N_0 is young-of-the-year abundance in September, E is total egg production for the year, and α and β are parameters. For details of this analysis see Reish (1985). Equation (1) is nonstochastic, but actual recruitment is highly random even with constant egg production (Nelson et al. 1977). A model fitting the random nature of the data is given by

$$(2)\quad N_0 = 1/(\alpha + \beta/E + \epsilon)$$

where ϵ is a normal random variable with mean 0 and standard deviation σ. The parameters α, β, and σ are fixed but ϵ varies between years. Menhaden data show that both mean recruitment and recruitment variability increase with E. Moreover, recruitment is positively skewed with occasional years of very large recruitment. A methodology developed in Carroll and Ruppert (1984) and applied there to menhaden data shows that equation (2) fits the stochastic behavior of the data. Alternative models, e.g. $N_0 = (1/(\alpha + \beta/E)) + \epsilon$, provide a less satisfactory fit. Since N_0 approaches $1/(\alpha + \epsilon)$ as E increases, the biological significance of model (2) is its suggestion that the environment's carrying capacity for menhaden varies annually through ϵ. This is reasonable, since estuarine areas certainly vary annually in the amount of food and habitat available.

Model (2) is not entirely satisfactory, since the denominator may be negative and, even when it is positive, N_0 is unbounded as the denominator approaches 0. Therefore, we decided to truncate the denominator in (2) so that it could never fall below $1/(2N_{0,\max})$ where $N_{0,\max}$ is the largest recruitment observed in the data. Thus, N_0 could never exceed $2N_{0,\max}$, which seemed like a reasonable restriction.

Statistical analysis of menhaden weight data (Reish et al. 1985) suggested that during the juvenile period prior to recruitment, growth is density-dependent, but growth after recruitment is independent of density. Female fecundity is also likely to be density-dependent, since egg production is size-dependent (Higham and Nicholson 1964).

Using menhaden length and weight data from 1965 to 1978, Reish et al. (1985) studied the relationship between weight at age and prerecruitment density of menhaden. It was found that average weight at age i, denoted by W_i, can be modeled as

$$(3)\quad W_i(N_0) = v_i N_0 + z_i$$

where N_0 is recruitment into the catchable stock; N_0 is used here as an index of prerecruitment abundance. The parameters v_i and z_i were estimated by least squares. The estimates of v_i were negative, so W_i is modeled as a linearly decreasing function of N_0. The adequacy of model (3) instead of a more complicated nonlinear model is due to the nature of the data: N_0 varies only moderately except for a few exceptional years and there is large amount of unexplained random variability in $W_i(N_0)$.

Equation (3) did not seem suitable for use when N_0 exceeds the largest value of recruitment observed in the data base. That is, we did not feel that extrapolating the linear function beyond the observed data was warranted. Instead we used (3) only for $N_0 \leq N_0^*$ where $N_0^* = 8.28 \times 10^9$. Parameter N_0^* is the estimated value of N_0 in 1976 when N_0 peaked for the years 1965–76. For $N_0 \geq N_0^*$ we used

$$(4)\quad W_i(N_0) = 0.25 W_i(N_0^*) \times \{\exp[-(4v_i/W_i(N_0^*))(N_0 - N_0^*)] + 3\}.$$

The function obtained by piecing together (3) and (4) is decreasing, continuously differentiable, and exponentially decreases to $(3/4)W_i(N_0^*)$ as N_0 increases beyond N_0^*.

Female egg production at age i was also modeled by equations of the form (3) and (4), but with W_i replaced by egg production at age i, called E_i here, and the estimates v_i and z_i replaced by

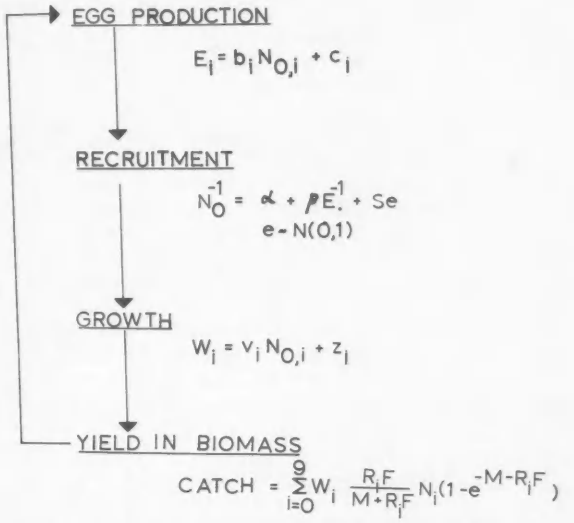

FIG. 1. Flowchart of a year of the stochastic simulation model with a constant fishing mortality rate policy in use. c_i is a constant contribution of eggs in age class i. b_i is the effect of density dependence on egg production (E). $N_{0,i}$ is the abundance prior to recruitment of a cohort age i. Likewise, z_i is a constant average weight (W) in age class i and v_i is the effect of density dependence on growth. The recruitment function is the inverse form of the Beverton–Holt equation ($s = \sigma$). R_i is the average instantaneous fishing mortality for years 1973–75 in age i divided by the average instantaneous fishing mortality for the same period in age 2. $R_i = 1$ when $i = 2$, $R_i < 1$ when $i < 2$, and $R_i \sim 1$ when $i > 2$.

estimates b_i and c_i obtained from the menhaden length data and the female fecundity–length relationship in Higham and Nicholson (1964).

Reish et al. (1985) estimated age-specific natural mortality for Atlantic menhaden using mark–recapture data. The estimates were adjusted for tag shedding, handling mortality, and the nonrecovery of tags by menhaden processing plants. The natural mortality rate is denoted by m.

Fishing mortality (F), of course, depended upon the harvesting policy, and this will be discussed in detail later. For each year, a policy determined, directly or indirectly, the instantaneous fishing mortality rate (F_2) and the exploitation fraction (μ_2) for age 2 menhaden. In the model, instantaneous fishing mortality rates for the other ages were determined so that the ratios of F_2 (for the constant F policy) or of μ_2 (for the constant catch and egg escapement policies) to the other age-specific rates were kept equal to the corresponding estimated ratios (called R_i for age $= i$) determined for the actual population between 1971 and 1975. Figure 1 illustrates a yearly cycle of the model with a fishing mortality rate policy in use. In Fig. 1, only the linear part of the egg production and growth function is illustrated. The catch equation in Fig. 1 is discussed below.

Utility Functions and Opportunity Losses

The utility functions in our study measured the value of a given amount of catch (Fig. 2). Past research on fishing policies normally considered the value of a catch as proportional to its biomass. Such a utility function is called "risk neutral" and is characterized by having a constant first derivative (or marginal utility). The use of utility functions in salmon management is discussed by Mendelssohn (1982), Hilborn and Walters (1977), and Keeney (1977).

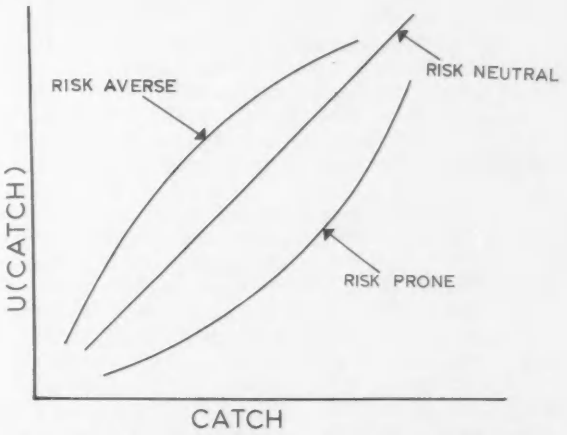

FIG. 2. Three examples of utility functions. U(CATCH) is the utility of the catch. Under the risk neutral utility, the value of the catch is proportional to its amount. The risk averse, risk neutral, and risk preferring (or prone) utility functions have decreasing, constant, and increasing marginal utility functions (first derivatives), respectively.

Most decision makers are thought to be normally "risk averse," i.e. to have decreasing marginal utilities. This would mean that a unit of biomass in a small catch is worth more than the same unit in a larger catch. This makes sense from the viewpoint of a fishery biologist, a fishery manager, or a commercial fisherman. When catch is an adequate index of abundance, then a fishery biologist would normally consider individual fish from small catches to have greater utility than those from larger catches. The "reproductive burden" per fish increases as the population size decreases, so that individual fish in a small population have more value in terms of replenishing the stock. At low population levels, the manager becomes extremely cautious concerning the collapse of the fishery and is more averse to risk. A commercial fisherman generally has more time and money invested per fish in smaller catches. For example, a scarcity of Atlantic menhaden will increase the search time and number of purse seine sets needed to fill a vessel (Nicholson 1971). Also, after a fisherman has caught enough to meet expenses and earn a reasonable profit, additional catches may have less value to him.

In decision problems, one must choose between many courses of action. In the present context, a decision is a choice of harvesting policy. Decision making involves risk because among the many possible "states of nature," we do not know the true one. If we ignore uncertainty about biological parameters, such as m or the parameters α, β, and σ in model (2), then in our situation the state of nature is the set of values of the random variables ϵ in (2). Later, when we consider uncertainty about parameters, the state of nature is the set of ϵ values and parameter values.

When one knows the probability of each possible state of nature, then it is possible to calculate the expected utility for any decision. If there is only a finite number, say k, of states of nature, then the expected utility of a decision is $\sum_{i=1}^{k} P_i U_i$, where P_i is the probability of state of nature i and U_i is the utility of state i given that particular decision.

With continuously distributed states of nature, the sum is replaced by an integral, which in our case cannot be analytically evaluated and must be approximated by an average over many Monte Carlo simulations. There is general agreement that when expected utilities are known, then the decision with the greatest expected utility should be chosen. In our case, expected utilities are known when we ignore uncertainty about biological parameters.

Often, however, one cannot objectively evaluate the probability distribution of the state of nature, and one is also unwilling to subjectively assess these probabilities. For us, this situation occurs when we include biological parameters as part of the state of nature. In this case there is no consensus on what decision is best, since there is no natural criterion, like expected utility, to optimize. In this case, it is worthwhile to calculate "opportunity losses." For a fixed state of nature, the opportunity loss for any decision is the difference between this decision's utility and the maximal utility over all possible decisions. Some decision makers would rule out decisions that can have a large opportunity loss, even if the probability of a large loss is unknown.

Opportunity loss is calculated for hypothetical populations in a number of papers (e.g. Walters and Ludwig 1981) but it is seldom called by its proper name; usually it is simply referred to as the difference between the optimal catch (the perfect information case) and the catch obtained from another given policy (the imperfect information case).

To find the true opportunity loss due to estimation error, we would need to know the "true value" for all parameters. Obviously we do not; we therefore approximate this loss by considering losses that would occur if the true value of a parameter were to lie a specified distance from our estimated value (also see below).

We focused upon harvesting over a 25-yr horizon, to ensure that a stochastic steady-state for the population would be reached. Simulation results for the constant F and egg escapement policies later showed that steady-state was reached by about 8 or 9 yr. This is not surprising, since menhaden of age 4 or greater are rare except under conditions of substantial underexploitation, females are mature by age 2 or earlier, and under the Beverton–Holt model, recruitment is virtually independent of egg production within the range seen in the simulations. Beddington and May (1977) have shown that an equilibrium distribution cannot exist for a constant catch policy.

The initial population vector was the estimated (by cohort analysis) population in 1976, the last year for which we had estimated abundances of all age classes. The criterion to be maximized was UT, defined as

$$(5)\quad \mathrm{UT} = E\left\{\sum_{i=1}^{25} U(C_i)\right\}$$

where E is mathematical expectation, c_i is the catch (in biomass) in the ith year, and U is a utility function. Notice that C_i depends upon the random recruitments in years 1 through i and upon the harvesting policy.

Because there was no single utility function that seemed natural, we worked with two rather different utilities, hoping that any reasonable utility function would fall between these extremes. The first utility function was $U(x) = x$, which had routinely been used in fisheries research and has a constant marginal utility. With this choice of U, our criterion became the maximization of total expected catch over 25 yr, with no attention paid to year-to-year variability of the catches. Such a utility is probably not completely desirable from several viewpoints but it represents one extreme.

The second utility function was $U(x) = \log(x + 1)$. Because it has a quickly decreasing derivative, $U'(x) = 1/(x + 1)$, this utility indicates extreme risk aversion. When this utility function is used, a harvesting policy with high year-to-year variability will have a lower expected utility (UT) value than a policy with the same total expected catch but with less variation in catch. Mendelssohn (1982) considered these two utility functions as the extremes (along a risk spectrum) for his study. An optimal policy under this utility must maximize the geometric mean of the catch. The use of the log utility is not related to any assumptions about the distribution of the catches, for example, that they are log-normal. The distribution of the catches depends upon the harvesting policy, and if an optimal policy is used then the catch distribution will, in fact, depend upon the utility function. Log utility is appropriate whenever there is a substantial aversion to risk.

Constant Catch Harvesting Policies

Perhaps the simplest harvesting policy is to take the same size catch, C^*, each year. When choosing C^*, one must balance the immediate reward of a large C^* with the possibility of extinction. Maximization of UT, as defined above, is one means of choosing this balance, since $C_i = C^*$ prior to extinction and $C_i = 0$ after extinction, if it occurs. Using stochastic approximation (Ruppert et al. 1984), we found that UT is maximized with C^* equal to 325 and 340 t for $U(x) = \log(x + 1)$ and $U(x) = x$, respectively. As a referee had remarked, the inclusion of discounting would cause the 340 value to increase, perhaps substantially. We suspect that the relative merits of the three types of harvesting policies would not be changed by discounting at usual rates. In a somewhat different model, Mendelssohn (1982) found that optimal harvesting strategies were relatively insensitive to the discount factor compared with the utility function.

In the context of simulation, "extinction" is biological extinction, but for the actual population we can think of extinction as collapse of the fishery. Because economic collapse is so drastic, instead of maximizing UT one might prefer to control the extinction probability. In Fig. 3 the curve labeled "no-phase-in policy" is a plot of the extinction probability against the attempted catch, e.g. 25 times C^*, the total catch if extinction does not occur. (The "phase-in" policy is discussed below.) The expected catch will, of course, be less than $25C^*$, but presumably only a policy with a small extinction probability would be contemplated, and then the difference between the expected catch and $25C^*$ would be small. When constructing Fig. 3 we ran 250 simulations for each point. The constant catch values ranged from 300 000 to 400 000 t in increments of 5000 t.

Although the 1976 population size is not small compared with recent historic values, it is smaller than equilibrium population sizes under harvesting policies discussed later. To determine whether constant catch policies would be more successful if the population was first allowed to increase, we defined a phase-in policy where the catch is C^* for years 4–25, but is only 25, 50, and 75% of C^* for years 1, 2, and 3, respectively. Thus, provided extinction does not occur, the total catch over 25 yr will be $23.5C^*$. In Fig. 3 we plotted the extinction probability against $23.5C^*$ for the phase-in policy. From this figure, we see that with an average yearly catch of 400 000 t (total catch = 10 million t) the extinction probability is almost 0.15 with phase-in,

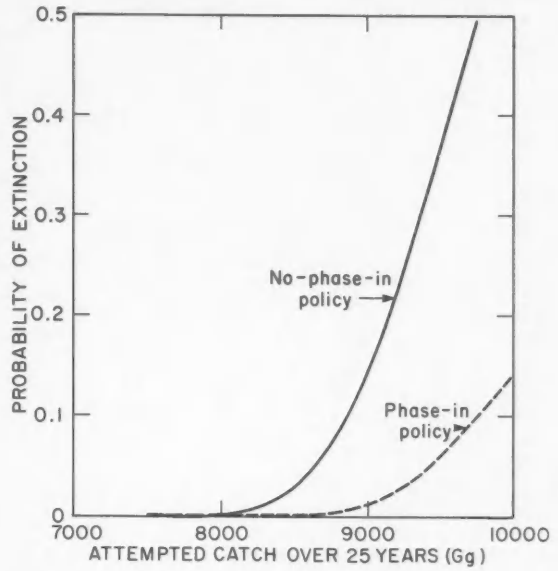

FIG. 3. Probability of extinction for the constant catch policies. Attempted catch is the total catch over 25 yr (thousands of metric tons = gigagrams) if extinction does not occur.

but almost 1.0 without. This comparison between the no-phase-in and phase-in policies, of course, depends heavily upon the initial conditions. If the initial population vector had been similar to the equilibrium distribution under optimal constant F policies (see below), then the phase-in and no-phase-in policies would almost certainly have had similar performances.

Our results are similar to the finding in Kirkwood (1981) that the probability of stock collapse over a fixed period of time decreases as catch is decreased. Theoretical analysis of a simpler model led Beddington and May (1977) to conclude that under a constant catch policy, population fluctuations increase with time, and extinction is inevitable.

Constant Fishing Mortality Rate Policies

For many fisheries models, a policy that keeps the instantaneous fishing mortality rate constant will produce higher catches than a constant catch policy. We will call a constant F policy one where the age 2 fishing mortality rate is fixed. For our model this implies that all other age-specific fishing mortality rates are constant; see the above description of the model. For populations with highly variable recruitment, a constant F policy, which adjusts catches to accommodate fluctuations in the population, seems preferable to constant catch policies.

We simulated the fishery 250 times for each value of F_2 from 0.1 to 4.0, incremented by 0.1, and plotted the Monte Carlo average utility as a function of F_2. Figure 4, the graph for the utility function $U(x) = x$ where catch = utility is a smoothing spline produced on the SAS/GRAPH software package (SAS Institute, Inc. 1981). The graph for $U(x) = \log(x + 1)$ is somewhat similar, and both have a maximum at about $F_2 = 0.8$. We estimate that during the mid-1970's, the menhaden fishery was operating with F_2 around 1.8, which is clearly suboptimal (Reish et al. 1985). In Fig. 5 we plotted the Monte Carlo average catch in biomass for years 1–25 for the $F_2 = 0.8$ policy. There

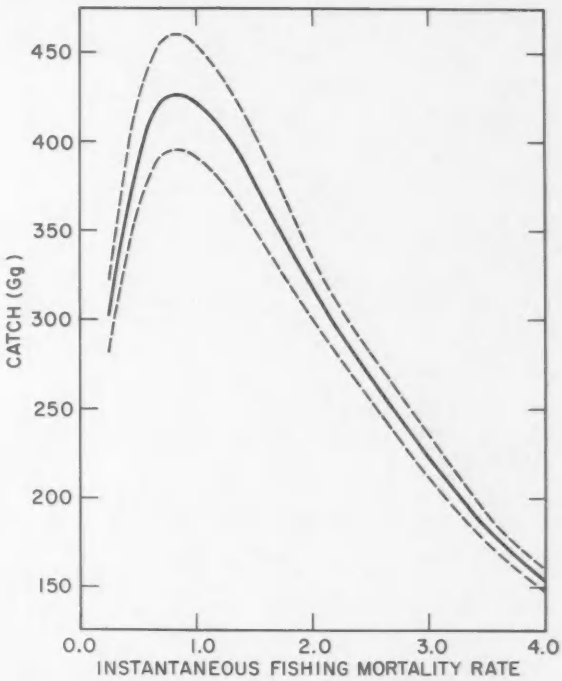

FIG. 4. Catch in biomass plotted against the yearly fishing mortality rate. The estimated mean annual catch and 95% confidence intervals for the mean are given.

were 250 simulations for each year. The actual average catches and a smoothing spline are plotted. Similar graphs show that population size, recruitment, and egg production each increase for 8–10 yr and then reach equilibrium.

Egg Escapement Policies

Among all harvest policies the constant escapement strategy of allowing an optimal amount of spawning to occur each year will maximize total catch in most autonomous population models (Clark 1976; Reed 1974; Mendelssohn and Sobel 1980). Such policies are termed "bang-bang" and they can result in a boom and bust type fishery with no catch when stocks are below optimal stocksize. For age-structured populations, the notion of optimal spawning escapement can be extended directly to a policy basing yearly catch upon potential egg production (defined as the combined egg production for all age classes that would be obtained if there was no fishing during that year). A flexible class of harvesting policies can be obtained by letting

$$(6)\quad C = A(E_p - B)^\theta \quad \text{if } E_p > B$$
$$= 0 \quad \text{if } E_p \leq B$$

where C and E_p are, respectively, catch and potential egg production in a given year and A, B, and θ are parameters.

If $\theta = 1$ and $B > 0$, then by choosing A properly one can keep actual egg production fairly constant (the so-called constant escapement policy) but not exactly constant, since egg production per unit biomass varies with the random age structure. The class of policies given by equation (6) is, in fact, much richer than the class of escapement policies, since both parameters A

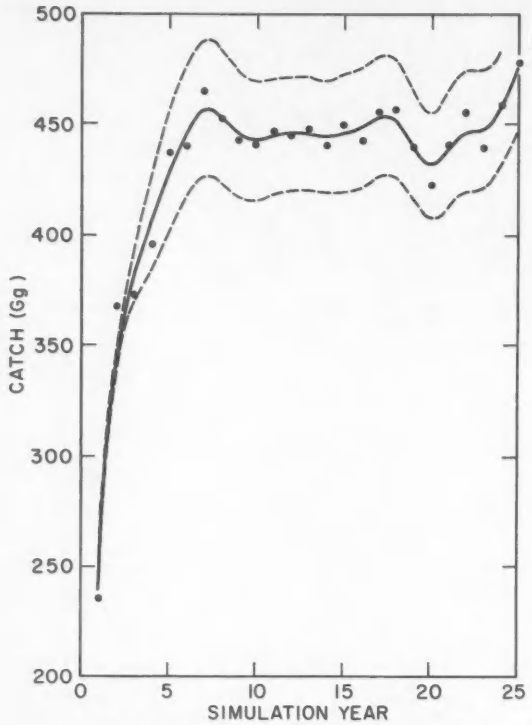

FIG. 5. Catch in biomass plotted against simulation year. The optimal constant fishing mortality rate ($F_2 = 0.8$) policy was in effect. The estimated mean annual catch and 95% confidence limits for the mean annual catch are given.

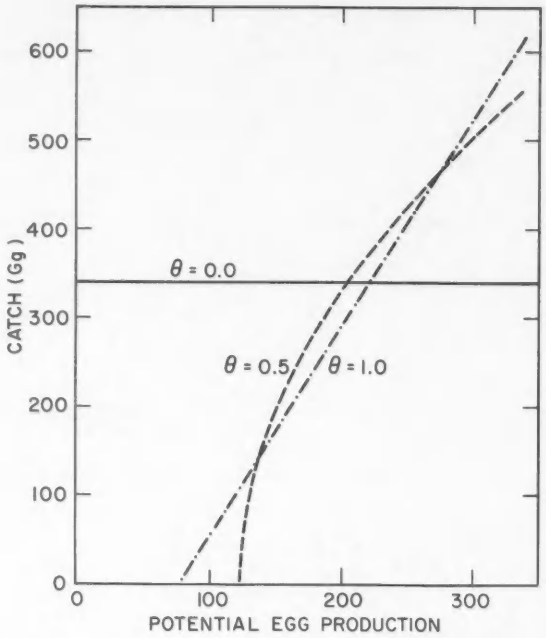

FIG. 6. Optimal catch functions for egg escapement policies when the utility function is $U(x) = x$. Potential egg production is in units of 10^{12} eggs.

and B are allowed to be specified. For example, by setting $B = 0$, we then obtain a policy similar to the constant F policy discussed earlier. By allowing $(-B) \to \infty$ and $A \to 0$ in such a way that AB = constant, we obtain a constant catch policy.

Letting $\theta = 1$ can result in great fluctuations in catches, whereas $\theta = 0$ results in constant catches. Having $0 < \theta < 1$ compromises between these extremes, so we investigated policies with $\theta = 1/2$ as well as with $\theta = 1$. The $\theta = 0$ policies have already been discussed. For each value of θ and for both $U(x) = x$ and $U(x) = \log(x + 1)$, we used stochastic approximation to find the values of A and B optimizing expected utility (Ruppert et al. 1985).

Figures 6 and 7 show the catch functions given by equation (6) with the optimal A and B. When $\theta = 1/2$, the optimal policy depends very little upon the choice of U, but if $\theta = 1$ then A and B are markedly different for the two utilities. When $U(x) = \log(x + 1)$, the optimal policy dampens fluctuations in catch size by having $B < 0$ and the slope A relatively small.

For each of the four optimal egg escapement policies ($\theta = 1/2$ or $\theta = 1$ and $U(x) = x$ or $U(x) = \log(x + 1)$) we plotted catch and population size (both in numbers of fish and biomass), recruitment, and potential and actual egg production against the simulation year. The four egg escapement policies and the $F_2 = 0.8$ policy behave somewhat similarly, but with a few notable differences. For the egg escapement policies, catch in biomass stabilizes at higher levels than for the $F_2 = 0.8$ policy. Also, the five policies have substantial differences in catch variability and in expected catch during years before equilibrium is reached. In

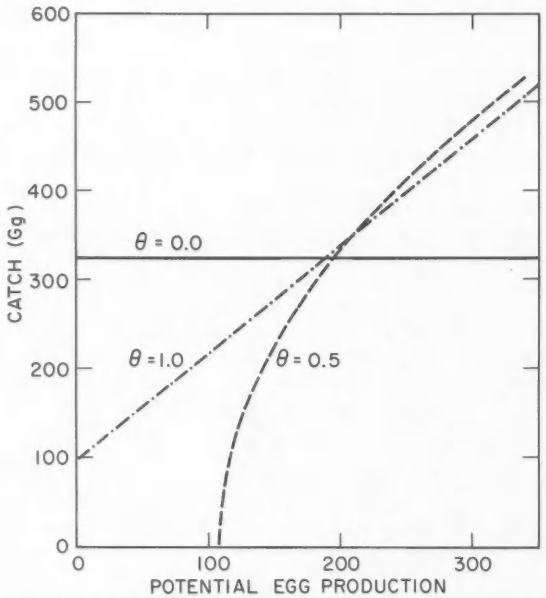

FIG. 7. Optimal catch functions for egg escapement policies when the utility function is $U(x) = \log(x + 1)$. Potential egg production is in units of 10^{12} eggs.

TABLE 1. Mean and standard deviation of annual catch (thousands of metric tons). In each row the top entry is the mean and the bottom entry is the standard deviation. The population is approximately in equilibrium by year 9. Years 1–8 show the approach to equilibrium.

Policy	Year 1	2	3	4	5	6	7	8	15
$F_2 = 0.8$	236	368	373	396	437	441	465	452	450
	1	12	79	186	235	221	257	240	220
$\theta = 1.0$	124	421	329	583	538	522	556	512	528
$A = 2.36$ Optimal for									
$B = 77.6$ $U(x) = x$	0	21	128	529	440	408	432	365	406
$\theta = 1.0$	256	355	327	446	484	499	524	513	527
$A = 1.21$ Optimal for									
$B = -80.5$ $U(x) = \log(x+1)$	0	11	64	227	248	236	261	238	244
$\theta = 0.5$	122	446	345	490	505	519	533	521	525
$A = 12.0$ Optimal for									
$B = 124$ $U(x) = x$	0	13	92	202	225	220	235	231	221
$\theta = 0.5$	166	405	339	464	491	509	526	518	527
$A = 11.0$ Optimal for									
$B = 108$ $U(x) = \log(x+1)$	0	12	78	179	203	202	215	212	203

Table 1, means and standard deviation of catch (in biomass) are given for years 1–8 and year 15, the latter being typical of years 9–25. The egg escapement policy which is optimal for $\theta = 1.0$ and $U(x) = x$ approaches equilibrium very quickly and has a highly variable equilibrium catch, compared with the four other policies, which are similar among themselves, except that the $F_2 = 0.8$ policy has a smaller expected equilibrium catch.

In order to compare the three types of policies, the expected total utilities as defined in equation (5) are given in Table 2 for the optimal constant catch (no-phase-in), constant F, and egg escapement policies. The expected utilities for the constant catch policy are slight overestimates. They are attained only if extinction does not occur, but the probability of extinction is about 0.05. For comparisons, the $U(x) = x$ utility is perhaps preferable. The three expected utilities are very similar when $U(x) = \log(x + 1)$, but it should be remembered that there is no fixed "zero point" for this utility; if the utility had been $U(x) = (\log(x + 1)) - 140$, then the expected utilities would have been dramatically different: 4.7, 9.7, and 13.1.

TABLE 2. Expected total utility for the three types of harvesting policies. The expected utilities for the constant catch policy is a slight overestimate, since it assumes that extinction does not occur.

Type of policy	Expected total utility $U(x) = x$	$U(x) = \log(x+1)$
Constant catch – no-phase-in	8 500	144.7
Constant F	10 779	149.7
Egg escapement	12 596	153.1

TABLE 3. Estimates of biological parameters used in the stochastic simulation model.

Parameter	Notation	Estimate	Standard deviation of estimate
Natural mortality rate	m	0.50200	0.0914
Constant in Beverton–Holt curve	α	0.00019750	0.000047
Slope in Beverton–Holt curve	β	3.17695	1.38619
Standard deviation in Beverton–Holt curve	σ	0.00012591	—

Risks due to Parameter Estimation Error

To assess risks due to estimation error, we estimated the standard errors for most estimates in the model and approximated the opportunity loss caused by this error. Because of the large data base available, growth parameters were estimated relatively precisely, so we concentrated on four other parameters: m, the instantaneous natural mortality rate, and the parameters α, β, and σ in the spawner–recruit equation (2).

The parameter σ^2 was estimated by the mean square for error from the least-squares regression of $1/N_0$ on $1/E$. Of course, E is estimated with error and this would cause σ to be overestimated, since variability in the estimation of E would be attributed to variability in recruitment. Errors in E also probably biased our estimates of α and β (Walters and Ludwig 1981), but lacking knowledge of the structure of the errors, we have not attempted bias corrections.

Estimates of m, α, β, and σ are given in Table 3. We calculated opportunity losses due to estimation errors of standard deviation in magnitude. Since m appears to be a crucial parameter, we also considered misestimation of m by two standard deviations. As σ is likely to be smaller than $\hat{\sigma}$, we calculated the opportunity loss if $\sigma = (0.7)\hat{\sigma}$. The value 0.7 was chosen simply because some definite value was needed. We did not know the real magnitude of overestimation of σ, but recruitment certainly does vary greatly, and we felt it unlikely that σ is much less than $(0.7)\hat{\sigma}$.

In Table 4 the calculations of opportunity losses are given for

TABLE 4. Calculation of opportunity losses. The upper value is the expected utility for the policy that is optimal for the changed value of the biological parameter. The middle value is the expected utility for the policy that is optimal for the parameters equal to the estimates given in Table 2. The lower value, the difference between the two, is the opportunity loss.

Change in parameters from estimates given in Table 2	$U(x) = x$		$U(x) = \log(x+1)$	
	$\theta = 1$	$\theta = 0.5$	$\theta = 1$	$\theta = 0.5$
None	12596.5	12501.5	153.145	153.250
	Same	Same	Same	Same
	0	0	0	0
m is 2 SD	8453.5	8267.7	143.407	142.973
above estimate	7732.8	7680.0	143.049	139.578
($m = 0.6848$)	720.7	587.7	0.358	3.395
m is 1 SD	10298.0	10158.6	148.260	148.327
above estimate	10078.6	9984.8	148.068	147.483
($m = 0.5934$)	219.4	173.8	0.192	0.844
m is 1 SD	15543.7	15530.1	158.492	158.714
below estimate	15236.0	15375.4	158.197	158.634
($m = 0.4106$)	307.7	154.7	0.295	0.080
m is 2 SD	19827.5	19654.1	164.223	164.872
below estimate	17956.6	18978.2	163.119	164.087
($m = 0.3192$)	1870.9	675.9	1.104	0.785
$\sigma = 0.7\hat{\sigma}$	10649.3	10642.6	150.353	150.429
($\sigma = 0.881392$)	10630.2	10637.6	150.254	150.247
	19.1	05.0	0.099	0.182
α is 1 SD	10199.4	10159.1	148.685	148.760
above estimate	10169.3	10154.5	148.578	148.502
($\alpha = 0.0002444$)	30.1	04.6	0.107	0.258
α is 1 SD	16040.8	15880.9	158.745	158.928
above estimate	16006.9	15831.7	158.698	158.788
($\alpha = 0.0001506$)	33.9	49.7	0.047	0.140
β is 1 SD	11962.8	11918.8	152.029	152.205
above estimate	11949.1	11900.2	151.928	152.083
($\beta = 4.5631$)	13.7	18.6	0.101	0.122
β is 1 SD	13377.1	13215.2	154.693	154.823
below estimate	13322.7	13149.4	154.397	154.452
($\beta = 1.7908$)	54.4	65.8	0.296	0.371

the four optimal egg escapement policies. To illustrate one calculation, suppose $U(x) = x$ and $\theta = 1$. To calculate the opportunity loss if m is two standard deviations above its estimate, the optimal values of A and B are calculated for $m = \hat{m}$ and $m = \hat{m} + 2s(\hat{m})$ (where $s(\hat{m})$ is the standard error or $\hat{m}$). From Table 4 we see that if $m = \hat{m} + 2s(\hat{m})$ and the values of A and B optimal for this m are used, then the expected utility (as estimated by simulation) is 8453.5. However, we believe that $m = \hat{m}$, so we would use the values of A and B optimal for $m = \hat{m}$. Here, the expected utility is 7732.8. The opportunity loss is $8453.5 - 7732.8 = 720.7$. This is the decrease in total utility (total catch over 25 yr) caused by using the policy optimal for $m = \hat{m}$ when, in fact, $m = \hat{m} + 2s(\hat{m})$. The opportunity loss is less than 10% of the expected utility, which seems small considering that an estimation error of two standard errors has only about a 5% chance of occurring.

Table 4 shows that opportunity losses are extremely small when $U(x) = \log(x + 1)$, and moderately small when $U(x) = x$. The worst cases are when m is misestimated.

A sensitivity analysis was also made. By sensitivity we mean the change in the expected utility of a fixed policy due to a change in a parameter. This can be large. For example, let $U(x) = x$ and $\theta = 1$ and consider the policy that is optimal if all parameters are equal to our estimates. The expected utility is 12596.5 if $m = m$ but only 7732.8 if $m = \hat{m} + 2s(\hat{m})$, a 39% change.

In summary, the small values of the opportunity losses show that estimation errors can only cause our egg escapement policies to be slightly suboptimal compared with what would be obtainable if the parameters were known, but estimation errors do prevent us from predicting average future catches with great accuracy.

Discussion and Conclusions

Our analysis suggests that Atlantic menhaden were much overexploited in the mid-1970's when the fishery was operating with F_2 around 1.8, more than twice the fishing mortality ($F_2 = 0.8$) we found to be optimal for maximizing either average catch or average logarithm of catch. That this relatively low F_2 was optimal is to a large extent due to increases in yield per recruit accrued by increasing mean age of the stock. Growth and natural mortality data show that biomass of a cohort peaks between ages 2 and 3 yr, whereas current fishing intensity allows few menhaden to survive even to age 3 yr.

Policies that allow for increases in the long-term harvest are numerous. Constant fishing mortality and egg escapement policies all work well. Constant catch policies where annual quotas remain fixed are not as desirable, because of high extinction probabilities when the quota exceeds 400 000 t. High extinction probabilities have been found in other constant catch models (Beddington and May 1977; Kirkwood 1981). Table 1 shows that catches are slightly lower with a constant fishing rate compared with egg escapement policies, with the highest total catch (Table 4) obtained by essentially a constant escapement policy. But the constant escapement policy has a higher standard deviation of catch than the constant fishing rate and the compromise ($\theta = 1/2$) policies. High catch variability for the constant escapement policy is well known in other population models (Allen 1973; Gatto and Rinaldi 1976). Our study of risk averse harvesting strategies (where we use a log utility function) gave some results that were surprising to us. The $\theta = 1$ policy did almost as well as the compromise $\theta = 1/2$ policy, whereas we originally thought the concave shape of a $\theta = 1/2$ curve would be far superior. Interesting as well was the lower opportunity loss for the $\theta = 1$ policy in seven of the nine cases considered in Table 4. We attribute the good results of the $\theta = 1$ policy to the fact that the two parameters A and B were allowed to be specified. This flexibility allowed the linear relationship between catch and potential egg production to give a flattened (reduced slope) line as compared with the line in our constant escapement policy (when catch is maximized). The resulting harvest policy is smoother with catches allowed for all stock sizes — a result also found in the study of a Ricker spawner–recruit curve by Mendelssohn (1982). A flattened linear relationship between catch and potential spawning is nearly the best possible log utility policy for some hypothetical populations (R. Hilborn and R. B. Deriso, unpubl. data).

We interpret our results as giving one clear advantage to management policies based on log utility. Long-term average catches are nearly maximized, while the fishery is much more stable (Table 1). The log utility policy for the $\theta = 1$ case seems especially realistic: the fishery is not completely shut down if

stocks are below some optimal size. The menhaden fishery has never been regulated and there is some strong resistance to management. A policy allowing complete shut down is unlikely to be accepted by fishermen. We encourage other researchers to present results for log utility objectives as well, so that we might better understand the benefits of proposed management policies.

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Biomass of Freshwater Crustacean Zooplankton from Length–Weight Regressions

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Culver, D. A., M. M. Boucherle, D. J. Bean, and J. W. Fletcher. 1985. Biomass of freshwater crustacean zooplankton from length–weight regressions. Can. J. Fish. Aquat. Sci. 42: 1380–1390.

We present equations for calculating dry weight from body length for 14 common and widely distributed taxa of crustacean zooplankters; these were generated by regression from weights of individuals chosen from the range of lengths observed for each taxon, usually three replicate weights at each of five lengths. We include regressions for ovigerous and nonovigerous cladocerans, plus nauplii, early copepodites, and adult males and females for the copepods (30 length–weight relationships) and individual weights for given stages of copepods. We calculated the seasonal variation in crustacean biomass for a station in the Bay of Quinte, Lake Ontario, and for each of the three basins of Lake Erie. Biomass was dominated by Cladocera for both lakes, with Copepoda predominant in the spring. Mean individual dry weights varied seasonally for all taxa, confirming previous findings.

Les auteurs présentent des équations pour le calcul du poids sec à partir de la longueur du corps de 14 taxons communs et cosmopolites de crustacés zooplanctoniques. Ces équations ont été obtenues par régression du poids d'individus choisis parmi la gamme de longueurs observées pour chaque taxon, en général trois réplications de poids à chacune des cinq longueurs. Sont aussi incluses des régressions pour les cladocères ovigères et non ovigères ainsi que les nauplius, les larves copépoditiques et les mâles et femelles des copépodes (30 relations longueur–poids) en plus du poids individuel de certains stades de ces derniers. La variation saisonnière de la biomasse de crustacés a été calculée à une station de la baie de Quinte (lac Ontario) et dans les trois bassins du lac Érié. Les cladocères constituaient la plus grande partie de la biomasse dans les deux lacs et, au printemps, les copépodes étaient les plus abondants. Le poids sec individuel Moyen variait selon la saison chez tous les taxons, ce qui confirme les découvertes antérieures.

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Major studies have been preformed in recent years on the abundance of North American crustacean zooplankton on the Great Lakes (e.g. Watson 1974, 1976; Patalas 1969, 1972; Carpenter et al. 1974; Davis 1969); however, there are relatively few that have included estimates of the biomass of individual species or of the entire assemblage. Accurate biomass estimates of zooplankters by species and developmental stage are necessary for many studies involved with modelling biological processes occurring in the pelagic zones of lakes. These include studies of secondary productivity (Bottrell et al. 1976; Culver and DeMott 1978), assimilation and excretion of nutrients such as nitrogen and phosphorus (Comita 1968; Hargrave and Geen 1968; Peters and Rigler 1973), the role of basic metabolic rates in survival times of zooplankters (Threlkeld 1976), and virtually all models of energy and carbon flux through the zooplankton (e.g. Scavia et al. 1976; Serruya et al. 1980).

Most biomass studies published to date are based on single estimates of the individual species weight (e.g. Wilson and Roff 1973; Watson and Carpenter 1974) because organisms that vary from 0.1 to 100 μg dry weight are difficult to weigh. The accuracy of biomass estimates calculated using a constant conversion factor from numbers to biomass is limited by the indeterminate growth of cladocerans and the numerous instars of copepods. The relative abundances of different stages and sizes of organisms change seasonally, and the size of mature organisms of both cladocerans and copepods varies within a sample and seasonally (Culver 1980; Deevey 1960).

Hawkins and Evans (1979) provided the first intensive published study to follow the biomass of individual taxa of Great Lakes zooplankton through time. Their approach involved picking out a representative subsample of individuals of each taxon and determining sample-specific average weights.

We use mathematical relationships between length and weight for a given series of samples to estimate the biomass of other samples using a length–frequency distribution. Not only is measurement of individuals during enumeration less tedious than picking, but the size–frequency data can be used for other purposes such as studies of population dynamics, secondary productivity, size-selective predation, etc. European researchers have performed careful studies of length–weight relationships for their common species (e.g. Dumont et al. 1975; Bottrelle et al. 1976), but only Rosen (1981) has published data for a collection of North American species.

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TABLE 1. Taxa of crustaceans included in this study and the date and site where specimens were collected. Bay of Quinte, Lake Ontario = BQ. Western Basin, Lake Erie, stations: PIB = Put-in-Bay; WB2 = (41°45′N, 82°55′W); WB3 = (41°45′N, 83°2′W). Central Basin, Lake Erie, station: CB = (42°15′N, 81°6′W).

Taxon	Collection site No.	Collection data
Ceriodaphnia lacustris Birge	BQ	21 August 1974
Daphnia galeata mendotae Birge	WB2	28 July 1976
Daphnia retrocurva Forbes	WB3	28 July 1976
Bosmina longirostris (O. F. Müller)	CB	30 August 1976
Eubosmina coregoni (Baird)	PIB	3 July 1976
Diaphanosoma leuchtenbergianum Fischer	CB	30 August 1976
Chydorus sphaericus (O. F. Müller)	BQ	21 August 1974
Leptodora kindtii (Focke)	WB2	28 July 1976
Calanoid nauplii	PIB	6 August 1976
Diaptomus copepodites I–IV	PIB	6 August 1976
Diaptomus minutus Lilljeborg	PIB	3 July 1976
Diaptomus oregonensis Lilljeborg	PIB	1 August 1976
Diaptomus siciloides Lilljeborg	PIB	6 August 1976
Cyclopoid nauplii	PIB	6 August 1976
Cyclops bicuspidatus thomasi S. A. Forbes	CB	30 August 1976
Cyclops vernalis Fischer	PIB	1 September 1976
Mesocyclops edax (S. A. Forbes)	CB	30 August 1976

The use of a set of general equations relating length to weight (usually in the form $W = aL^b$ where a and b are fitted constants, W is the mass in micrograms, and L is the length in millimetres) has certain problems and limitations. The equation can only estimate weights for organisms whose lengths are within the range of measurements included in the regression. The method assumes that the relationship between weight and length is insensitive to changes in food, temperature, reproductive state, cyclomorphosis, and sampling location. More generally, one assumes that the relationship between size and weight is extremely important to planktonic organisms, because of its effect on sinking rates, and that while environmental change may affect length or the time required to grow to a given length, it will not affect the relationship between length and weight.

Before an equation produced from a single study is used for other times of the year or even other lakes, comparisons of length–weight regressions for the same species from different times of the year and from lakes of differing productivity and temperature regimes will be needed. As a first step, we present in this paper regressions calculated from lengths and weights of six copepod and eight cladoceran zooplankton taxa collected from single sites and seasons from Lakes Erie and Ontario by Boucherle (1977) and us. We determined statistically to what extent different species and developmental stages fall on the same regression lines, and the effects of sexual dimorphism and cyclomorphism on length–weight relationships. The regression equations are then used to calculate seasonal variation in biomass for a series of stations in Lakes Erie and Ontario, and the results are used to examine seasonal variation in individual species weight for the same samples.

Materials and Methods

All zooplankters weighed during this study were collected from the Western and Central Basins of Lake Erie during July, August, and September 1976 or from the Bay of Quinte, Lake Ontario, 1974 (D. A. Culver, unpubl. data) (Table 1). Zooplankters were anesthetized with carbonated water (Gannon and Gannon 1975) and preserved in lake water plus 4% formaldehyde, or 4% formaldehyde with 40 g sucrose/L added (Central Basin and Bay of Quinte samples only) to prevent "ballooning" of cladoceran carapaces (Haney and Hall 1973). Whether the use of sucrose and formalin for some of our cladoceran samples will alter the weights obtained is not known. Certainly the length measurements on sucrose + formalin-preserved animals are more accurate, because "ballooning" of the carapace grossly distorts the body shape. Most of our animals were weighed within a few months of preservation, but those from the Bay of Quinte had been preserved for 3 yr. Dumont et al. (1975) have suggested that formalin-preserved zooplankton were suitable for biomass determination long after preservation due to the rapid fixation of the animals by formalin, and the lack of weight loss over long periods. They estimate losses due to storage as not exceeding 5–10%.

Zooplankters were sorted by taxon, and each taxon was further sorted by length, sex, and reproductive condition (females) with a dissecting microscope using magnifications of up to 200 diameters. Each plankter was measured with an ocular micrometer according to its body shape (Fig. 1). To minimize variance in the regressions, we selected individuals that were the same length to the nearest ocular micrometer unit (1 unit = 20 μm at 100×) to constitute one sample. For the cyclomorphotic forms *Daphnia retrocurva* and *D. galeata mendotae*, we measured both from the anterior edge of the eye to the base of the tail spine (defined as standard length in this study) and from the anterior margin of the helmet to the base of the tail spine (defined as total length in this study). Because some investigators have measured copepods to the base of the caudal spines, we have compared this measure with our typical measure to the base of the caudal rami for all six species.

Sorted zooplankters were washed three times for 30 min in distilled water to remove preservative and transferred to a dried and tared platinum weighing pan with an Irwin loop. Weighing pans containing zooplankters were placed in a drying oven at 60°C for 2 h. Drying was continued in a desiccator over calcium chloride for 24 h before weighing the samples on a Cahn Gram

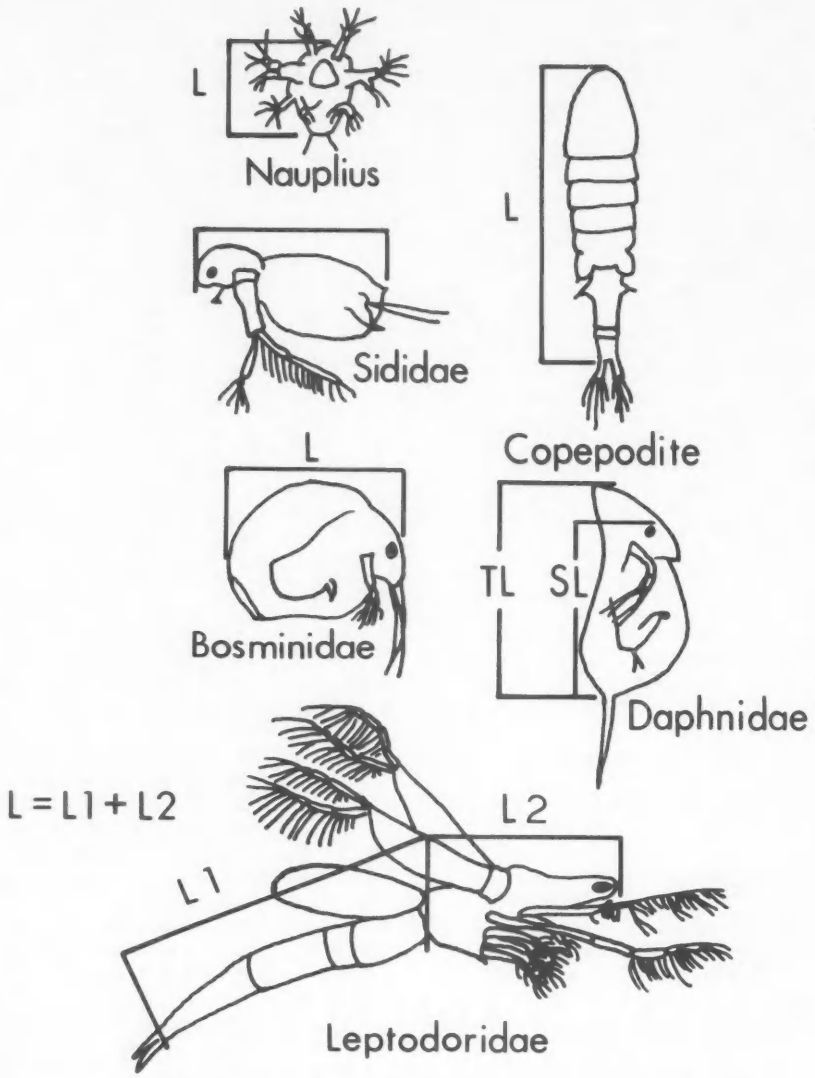

FIG. 1. Method of measuring length for the major groups of crustacean taxa.

Electrobalance. For a given taxon, at least three samples for the same length were weighed. The number of zooplankters used per weighing varied according to taxon and length, but sufficient organisms were added to achieve a minimum net weight of about 5 μg. Usually 10–20 animals were grouped per weight, but for nauplii, 40 animals were routinely used.

The values of a and b in the exponential equation were computed for each taxon by linear regression using the formula $\log W = \log a + b \log L$. Zooplankters of a given taxon used to calculate a regression equation were collected at the same time and sampling site (Table 1). Analysis of variance was performed on each regression to determine whether the slope of the line (value of b) was significantly different from zero. Length–weight regressions for various taxa were compared using small-sample t-tests for differences among slopes and for differences among intercepts (Kleinbaum and Kupper 1978). Two regressions were taken as different if either the slope or the intercept (or both) were different with a significance level of 0.05 (two-tailed test). We used the tabulated value for a significance level of 0.02 to account for the multiple comparisons of slope and intercept for each pair of regression equations. No attempt was made to correct further for multiple comparisons among species. Comparison of regressions with those in the literature was done by looking for overlap in confidence levels about slopes or intercepts, since the literature sources included no raw length–weight data.

Seasonal variation in crustacean zooplankton biomass in Lake Erie was examined for a series of samples collected in 1970 by the Canada Centre for Inland Waters (CCIW) in a series of 10 synoptic cruises to 30 stations. Abundances for the samples were provided by N. H. F. Watson, CCIW, Burlington, Ont., Canada. A proportional frequency distribution for these samples

was constructed after measuring 30 or more individuals of each species and stage. This was in turn multiplied by the abundance values and by the size-specific weight calculated for that length using our length–weight equations. These results were then summed and expressed as total cladoceran or copepod biomass per cubic metre. Results for the 4 stations from the Western Basin were averaged, as were the 18 stations in the Central Basin and 8 stations in the Eastern Basin. A similar process was used for data from the Bay of Quinte, Lake Ontario, for April–September 1976 (D. A. Culver, unpubl. data). Seasonal variation in average individual weights for each species was obtained for both the 1970 Lake Erie and 1976 Bay of Quinte samples by dividing the total biomass for each species and stage in a sample by its numerical abundance in the sample.

Results and Discussion

We obtained length–weight data for six copepod and six cladoceran taxa from Lake Erie. Two other cladocerans of interest (*Chydorus sphaericus* and *Ceriodaphnia lacustris*) were rare in our samples, so we obtained organisms from August 1974 samples from the Bay of Quinte, Lake Ontario. It is unknown what effect, if any, this will have on the estimation of biomass for organisms of these species from Lake Erie.

The copepod weights taken for a variety of sizes and instars showed that the largest growth increments occurred between CV and CVI (Table 2). The CVI (adult) individuals showed the well-known sexual dimorphism, but the length and weight variability in adults of a given sex in one sample was surprisingly high. The largest adult individuals were much longer than the smallest mature individuals of the same sex, and as much as 2.5 times as heavy.

Length–Weight Regressions

The regressions were all highly significant at least at the 5% significance level for both copepods and cladocerans; thus, values of a and b from the regressions of copepods and cladocerans (Table 3) are good representations of the length–weight relationships for these taxa. The slopes of all regression lines (b in Table 3) were statistically different from zero ($p < 0.01$ using F test and one-way ANOVA).

Combination of Regressions Among Species

Previous work has examined the suitability of combining data among species to generate a regression that would represent, for example, Cyclopoida, Calanoida, or Daphnidae, to simplify the size–frequency analyses required to generate biomass estimates. We tested the overlap between regressions for our various taxa to determine whether such combining was justified. Various combinations of regression equations for cladoceran species (Table 4) were not significantly different at the 0.05 significance level, but *D. retrocurva* was different from all other cladoceran taxa, including *D. g. mendotae*, which it closely resembles. Accordingly, one cannot merge *D. retrocurva* and *D. g. mendotae* regressions. Furthermore, care must be exercised to avoid confusing the two species in size–frequency determinations for estimating biomass. *Eubosmina coregoni* and *Bosmina longirostris* were not significantly different, so taxonomic separation of these two species is less critical in biomass determinations. Other cladoceran species varied in overlaps, but the lack of taxonomic difficulties among them argues in favor of using the species-specific regressions.

TABLE 2. Lengths and dry weights for selected copepod stages and taxa in Lake Erie (n = number of animals; the number of weight determinations was variable).

Taxon and stage	Length (mm)	Mean weight (μm)	n
Cyclopoid nauplii			
NI	0.144	0.096	120
NII	0.162	0.160	120
NIII	0.198	0.200	120
NIV	0.234	0.203	120
NV	0.270	0.382	120
NVI	0.315	0.405	80
Cyclops bicuspidatus thomasi			
CI	0.434	0.97	45
CII	0.471	1.30	15
CIII	0.525	1.50	30
CIV	0.597	2.73	17
CV	0.706	2.95	20
CVI females	0.724–0.905	3.4–4.9	67
Eggs		0.168	306
Cyclops vernalis			
CI	0.326	0.33	45
CII	0.380	0.80	44
CIII	0.416	0.83	45
CIV	0.489	1.07	45
CV	0.543	1.43	45
CVI females	0.597–1.086	1.8–8.3	127
CVI males	0.597–0.724	1.5–3.1	70
Mesocyclops edax			
CI	0.507	0.97	30
CII	0.579	1.20	30
CIII	0.634	1.77	30
CIV	0.670	2.73	30
CV	0.742	2.40	30
CVI females	0.778–1.050	3.8–7.2	120
Eggs		0.175	300
Calanoid nauplii			
NI	0.108	0.067	120
NII	0.162	0.147	120
NIII	0.198	0.170	120
NIV	0.243	0.280	120
NV	0.288	0.397	120
NVI	0.342	0.450	120
Diaptomid copepodites			
CI	0.362	0.83	45
CII	0.434	1.13	45
CIII	0.561	1.30	20
CIV	0.634	2.33	20
Diaptomus minutus			
CV	0.652	1.47	30
CVI females	0.706–0.851	1.97–3.55	102
Diaptomus oregonensis			
CV	0.724	3.23	30
CVI females	0.760–1.176	3.84–8.66	104
CVI males	0.778–0.815	4.33–4.80	50
Eggs		0.214	301
Diaptomus siciloides			
CVI females	0.959–1.176	4.75–10.5	77
CVI males	0.724–1.032	3.45–7.47	110

TABLE 3. Results of length–weight regressions. a, b = constants in $W = aL^b$; var L = variance of lengths; RMS = residual mean square; R^2 = coefficient of determination.

Taxon	Range in lengths (mm) (no. of weights)	$b \pm 95\%$ c.l.	a	var L	RMS	R^2
Calanoid nauplii NI–NVI	0.108–0.342 (18)	1.7064±0.1259	3.0093	0.1538	0.0035	0.98
Diaptomus copepodites I–IV	0.362–0.634 (18)	1.7034±0.1342	4.5921	0.5484	0.0038	0.98
Diaptomus minutus Lilljeborg						
Nonovigerous females CV–CVI	0.652–0.851 (15)	3.8564±0.0916	7.3614	0.0094	0.0018	0.99
Diaptomus oregonensis Lilljeborg						
Males CV–CVI	0.724–0.815 (7)	2.3482±0.1401	7.7416	0.0024	0.0545	0.93
Nonovigerous females CV–CVI	0.724–1.176 (15)	1.9604±0.0797	6.1927	0.0328	0.0014	0.99
Diaptomus siciloides Lilljeborg						
Nonovigerous females CVI	0.959–1.176 (14)	3.8498±0.0451	5.8853	0.0078	0.0004	0.99
Ovigerous females CVI	0.996–1.267 (14)	2.3701±0.0924	2.5344	0.0055	0.0018	0.98
Males CVI	0.724–1.032 (14)	2.6484±0.0865	7.8273	0.0172	0.0016	0.98
Cyclopoid nauplii NI–NVI	0.144–0.315 (18)	1.6349±0.2523	2.5968	0.0797	0.0142	0.99
Cyclops bicuspidatus thomasi S. A. Forbes						
Nonovigerous females CI–CVI	0.434–0.905 (18)	1.9347±0.1719	5.6713	0.0686	0.0066	0.99
Cyclops vernalis Fischer						
Nonovigerous females CI–CVI	0.326–1.086 (28)	2.5563±0.1975	7.0729	0.1724	0.0092	0.99
Males CI–CVI	0.326–0.724 (22)	2.5320±0.2264	6.8636	0.0730	0.0118	0.99
Mesocyclops edax (S. A. Forbes)						
Nonovigerous females CI–CVI	0.507–1.050 (26)	2.8945±0.2057	6.6586	0.0544	0.0099	0.99
Daphnia galeata mendotae Birge						
Nonovigerous females						
Standard length	0.362–1.810 (28)	1.5302±0.1963	10.9317	0.3137	0.0091	0.99
Total length	0.471–2.172 (28)	1.5644±0.2156	7.4997	0.2964	0.0110	0.99
Ovigerous females						
Standard length	0.905–1.629 (13)	1.6626±0.0130	16.1499	0.0193	0.0001	0.97
Total length	1.176–1.991 (13)	2.0317±0.0074	8.9451	0.0125	0.0001	0.97
Daphnia retrocurva Forbes						
Males, total length	0.495–0.594 (11)	3.8329±1.606	23.0471	0.1867	0.0260	0.93
Nonovigerous females						
Standard length	0.326–1.448 (24)	2.7552±0.1593	7.5484	0.2063	0.0050	0.99
Total length	0.398–1.810 (24)	2.6807±0.1402	3.7847	0.2188	0.0046	0.99
Ovigerous females						
Standard length	0.905–1.629 (14)	2.4502±0.0704	8.9561	0.0462	0.0010	0.99
Total length	1.176–1.991 (14)	2.7601±0.0933	4.2597	0.0359	0.0018	0.99
Bosmina longirostris (O. F. Müller)						
Nonovigerous females	0.217–0.434 (15)	2.2291±0.0870	17.7369	0.0316	0.0018	0.98
Ovigerous females	0.326–0.416 (15)	1.7892±0.0750	7.8565	0.0108	0.0012	0.99
Eubosmina coregoni (Baird)						
Nonovigerous females	0.272–0.543 (17)	2.3371±0.1451	21.9128	0.0479	0.0046	0.96
Ovigerous females	0.362–0.634 (14)	2.3321±0.0899	27.0090	0.0259	0.0017	0.98
Diaphanosoma leuchtenbergianum Fischer						
Nonovigerous females	0.313–0.525 (15)	1.0456±0.0617	5.0713	0.0313	0.0008	0.97
Leptodora kindtii (Focke)						
Nonovigerous females	2.268–6.804 (17)	1.8730±0.1295	1.5605	0.1774	0.0037	0.99
Chydorus sphaericus (O. F. Müller)						
Nonovigerous females	0.219–0.310 (8)	1.9796±0.2213	14.0793	0.0227	0.0014	0.99
Ceriodaphnia lacustris Birge						
Nonovigerous females	0.329–0.548 (13)	1.9763±0.2962	4.0216	0.0232	0.0066	0.99

Similarly, we found (Table 4) significant differences between regressions for *Cyclops bicuspidatus thomasi* copepodites and both those of *C. vernalis* and *Mesocyclops edax*. *Cyclops bicuspidatus thomasi* and *C. vernalis* are moderately difficult to separate taxonomically, but significant errors in biomass estimates will result from use of a merged group of measurements. Our adult calanoid regressions showed little overlap either, further suggesting the necessity of identification to the species

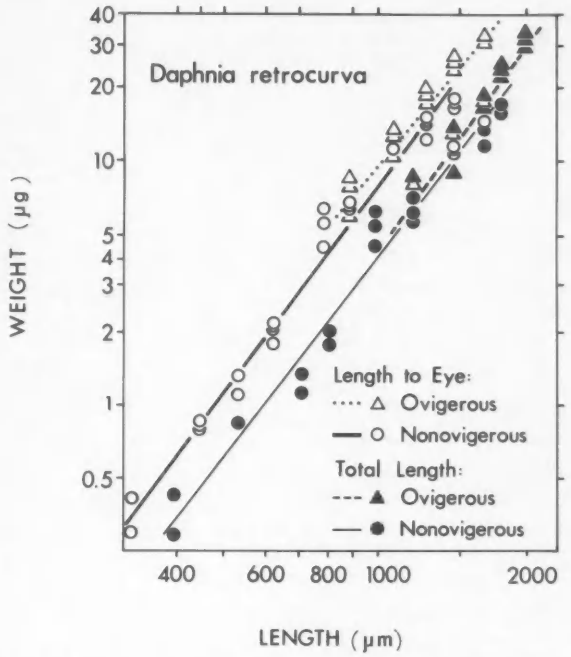

FIG. 2. Comparison of length–weight regressions based on total length (including the helmet) and on "standard length" (to the eye) for *Daphnia retrocurva* from Western Lake Erie.

level. The lack of significant difference between the regressions for cyclopoid and calanoid nauplii means that errors in separation between these taxa will not be a problem. Because the early copepodites (CI–CV) for the three cyclopoid taxa and *Diaptomus* spp. were not significantly different either, errors in identification of juvenile stages will not affect biomass determinations.

Sexual Dimorphism

Sexual dimorphism is well known in the plankton, but in our data this was more a result of differences in ranges of length than in body proportions for *Diaptomus oregonensis*, *D. siciloides*, and *C. vernalis* (Table 4). For all three cases, the range of male lengths was distinctly shorter than for the females of the same species, but the male and female data fell on the same regression line. We will need more data on males to determine whether this is a general result. The lines were different for the sexes in *D. retrocurva* (Table 4).

Developmental Stages

We merged the data for CI–CV and CVI for the three cyclopoid taxa, since in each case the regression lines were identical (Table 4). There was a distinct shift in body proportions from nauplii to adult copepodites in these taxa, however, so a separate line is still required for nauplii. Taxonomic difficulties precluded separating the calanoid copepodites by species until CV, and there is only fair agreement between the copepodite line and the CV–CVI lines. The nauplii did not differ significantly from the copepodites, so one could construct a combined line for them if desired. The values for *a* and *b* for the line are 5.04359 and 1.99585, respectively, and it behaves exactly like the *Diaptomus* CI–CIV line relative to the CV–CVI regressions.

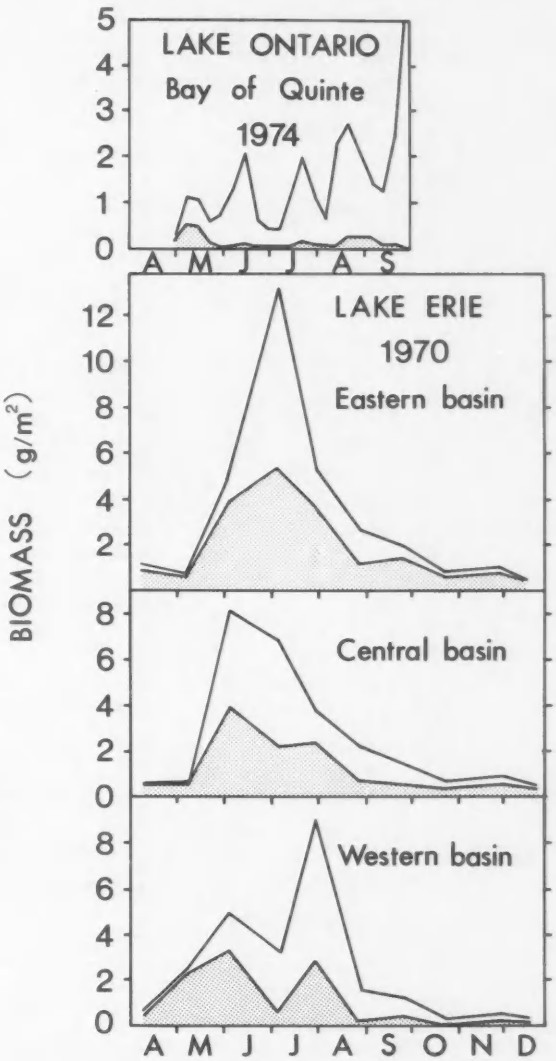

FIG. 3. Seasonal variation in crustacean dry weight biomass in Lakes Erie and Ontario. The contribution of Copepoda is represented by the shaded area.

Reproductive State

Except for *D. siciloides*, ovigerous animals' length–weight relationships were not different from those of nonovigerous animals (Table 4). The nonovigerous females samples certainly included some individuals with eggs in the ovaries; nevertheless, if nonovigerous regressions are to be used for ovigerous individuals, egg weights should be added. Variation in cladoceran egg size throughout the year (Kerfoot 1974) and in neonate size (Culver 1980) may also affect biomass estimates. In the biomass estimates for Lake Erie and Lake Ontario discussed below, we have used the nonovigerous regression and added for

TABLE 4. Statistical comparisons among regressions from Table 3 by species, sex, developmental stage, reproductive condition, and method of measurement (*Daphnia*). Taxa whose length–weight regression line is significantly different from those in the first column are placed in column 2 followed by the regression coefficient(s) that was(were) different (a = intercept, b = slope).

Taxon	Different	Not different
	By species	
Daphnia retrocurva	*Bosmina* (*a*), *Ceriodaphnia* (*a*), *Chydorus* (*a*), *D. g. mendotae* (*a*,*b*), *Diaphanosoma* (*b*), *Eubosmina* (*a*)	None
Diaphanosoma leuchtenbergianum	*Bosmina* (*a*,*b*), *Ceriodaphnia* (*a*,*b*), *Chydorus* (*b*), *D. retrocurva* (*b*), *Eubosmina* (*a*,*b*)	*D. g. mendotae*
D. g. mendotae	*Ceriodaphnia*, *D. retrocurva*, *Eubosmina* (*a*,*b* for all)	*Bosmina*, *Chydorus*, *Diaphanosoma*
Ceriodaphnia	*D. g. mendotae* (*a*,*b*), *D. retrocurva* (*a*), *Diaphanosoma* (*a*,*b*)	*Bosmina*, *Chydorus*, *Eubosmina*
Eubosmina	*D. g. mendotae* (*a*,*b*), *D. retrocurva* (*a*,*b*), *Diaphanosoma* (*a*,*b*)	*Bosmina*, *Chydorus*, *Ceriodaphnia*
Bosmina	*D. retrocurva*, *Diaphanosoma* (*a*,*b* for both)	*Ceriodaphnia*, *Chydorus*, *D. g. mendotae*, *Eubosmina*
Chydorus	*D. retrocurva* (*a*), *Diaphanosoma* (*b*)	*Bosmina*, *Eubosmina*, *D. g. mendotae*, *Ceriodaphnia*
Cyclops bicuspidatus thomasi CI–CVI♀	*C. vernalis* CI–CVI♀ (*b*), *Mesocyclops edax* CI–CVI♀ (*b*)	
C. vernalis CI–CVI♀		*M. edax* CI–CVI♀
Diaptomus oregonensis CV–CVI♂		*D. siciloides* CV–CVI♂
D. minutus CV–CVI♀	*D. oregonensis* CV–CVI♀ (*b*), *D. siciloides* CV–CVI♀ (*a*)	
D. siciloides CV–CVI♀	*D. oregonensis* CV–CVI♀ (*b*)	
C. b. thomasi CI–CV		*C. vernalis* CI–CV, *M. edax* CI–CV
C. vernalis CI–CV, CVI♀		*M. edax* CI–CV, CVI♀
Diaptomus spp. CI–CIV		*C. b. thomasi* CI–CV, *C. vernalis* CI–CV, *M. edax* CI–CV
Cyclopoid nauplii NI–NVI		Calanoid nauplii NI–NVI
	By sex	
D. retrocurva ♀	*D. retrocurva* ♂ (*a*)	
D. siciloides CV–CVI♀		*D. siciloides* CV–CVI♂
D. oregonensis CV–CVI♀		*D. oregonensis* CV–CVI♂
C. vernalis CVI♀		*C. vernalis* CVI♂
	By developmental stage	
Cyclopoid nauplii NI–NVI	*M. edax* CI–CVI♀ (*a*,*b*), *C. vernalis* CI–CVI♀ (*a*,*b*)	*M. edax* CI–CV, *C. vernalis* CI–CV, *C. b. thomasi* CI–CV, CI–CVI♀
C. vernalis CI–CV		*C. vernalis* CVI♀, CVI♂
C. b. thomasi CI–CV		*C. b. thomasi* CVI♀
M. edax CI–CV		*M. edax* CVI♀
Calanoid nauplii		*Diaptomus* spp. CI–CIV

TABLE 4. (*Concluded*).

Taxon	Different	Not different
Diaptomus spp. CI–CIV	*D. siciloides* CV–CVI♀ (*b*), *D. minutus* CV–CVI♀ (*a*, *b*)	*D. oregonensis* CV–CVI♂, *D. siciloides* CV–CVI♂, *D. oregonensis* CV–CVI♀
	Ovigerous vs. nonovigerous	
D. retrocurva (ovigerous)		*D. retrocurva* (nonovigerous)
D. g. mendotae (ovigerous)		*D. g. mendotae* (nonovigerous)
Eubosmina (ovigerous)		*Eubosmina* (nonovigerous)
Bosmina (ovigerous)		*Bosmina* (nonovigerous)
D. siciloides CVI♀ (ovigerous)	*D. siciloides* CVI♀ (nonovigerous) (*a*, *b*)	
	Cyclomorphosis	
D. retrocurva total length: nonovigerous ovigerous	*D. retrocurva* standard length: nonovigerous (*a*) ovigerous (*a*)	
D. g. mendotae total length: nonovigerous ovigerous	*D. g. mendotae* standard length: nonovigerous (*a*)	*D. g. mendotae* standard length: ovigerous

each egg or embryo a weight equivalent to the smallest nonovigerous individuals (5th percentile) occurring in that sample.

Cyclomorphosis

Cyclomorphosis does not significantly affect the length–weight regressions of nonovigerous individuals of *D. retrocurva* or *D. g. mendotae* for a sample collected on 28 July 1976. Regression equations for total length and standard length (measured from the eye to the base of the caudal spine) of *D. g. mendotae* had the same slope (Fig. 2) ($T = 0.31$, $p > 0.35$), although the intercepts were of course altered. For *D. retrocurva*, slopes again did not differ ($T = 0.71$, $p > 0.35$). This suggests that no bias is introduced by use of total length, even though we would expect the younger individuals to have more pronounced helmets than the older ones at this time of year. While we might have observed a difference had we taken samples from various times in the year, cyclomorphosis biases should be noticed in late July if at all. The intercepts for total and standard length were not quite different ($T = 2.021$, $t_{0.975,20df} = 2.086$) for ovigerous individuals of *D. g. mendotae* probably because variable numbers of eggs carried by these females increased the variance of the weights for a given length.

We found the ratio of copepod lengths measured to the base of the caudal rami (Fig. 1) to lengths measured at the end of the caudal rami to be quite invariant over a broad range of copepod sizes within a species, but the ratio did vary among species:

Taxon	Ratio of lengths (*n*)
Diaptomus copepodites I–IV	0.923±0.021 (23)
Diaptomus minutus	0.917±0.023 (19)
Diaptomus oregonensis	0.927±0.011 (21)
Diaptomus siciloides	0.937±0.011 (27)
Cyclops bicuspidatus thomasi	0.845±0.026 (29)
Cyclops vernalis	0.888±0.017 (30)
Mesocyclops edax	0.923±0.017 (32)

Measurement to the base of the caudal rami avoids measurement differences caused by changes in the angle of the caudal rami to the long axis of the body in preserved specimens.

Comparison with Literature Results

Comparison of our results with other studies remains difficult. There are no other published length–weight regressions for zooplankton from the Great Lakes, and the excellent work by Dumont et al. (1975) and Bottrell et al. (1976) was done on European species, although *C. sphaericus* and *B. longirostris* are common to both continents. Differences in technique and units of measurement complicate comparisons. We measured our animals and performed the regressions in micrometres, but converted the equations to millimetres to make the coefficients (*a*) more manageable. For $W = a(L \times 10^c)^b$, where 10^c is the conversion factor from micrometres to millimetres (10^3), the result may be written $W = a10^{cb}L^b$. The equation for calanoid nauplii is thus $W(\mu g) = 2.287 \times 10^{-5}\, L(\mu m)^{1.7064} = (2.287 \times 10^{-5})\ (10^{3\times 1.7064})\ L(mm)^{1.7064} = 3.0093\ L(mm)^{1.7064}$. This allows comparison of data for literature expressed in other units. For example, table 2 in Dumont et al. (1975) would have been more useful if equations had all been converted to micrograms and millimetres.

We found good agreement between our regression for nonovigerous *B. longirostris* with that from the River Thames in Bottrell et al. (1976). The confidence limits for the slopes overlapped (2.2291 ± 0.087 vs. their 2.5294 ± 0.57) and the values of the intercepts (log *a*) were close as well (2.875 vs. 2.7116). Other comparisons among our data and those of Bottrell et al. (1976) for the same taxa (*C. vernalis* copepodites V–VI) or related taxa (*Ceriodaphnia lacustris* vs. *C. quadrangula*, *Diaphanosoma leuchtenbergianum* vs. *D. brachyurum*, and *D. g. mendotae* or *D. retrocurva* vs. *Daphnia* spp.) showed significant differences among slopes, intercepts, or both. *Chydorus sphaericus* in our study was much heavier than those reported by

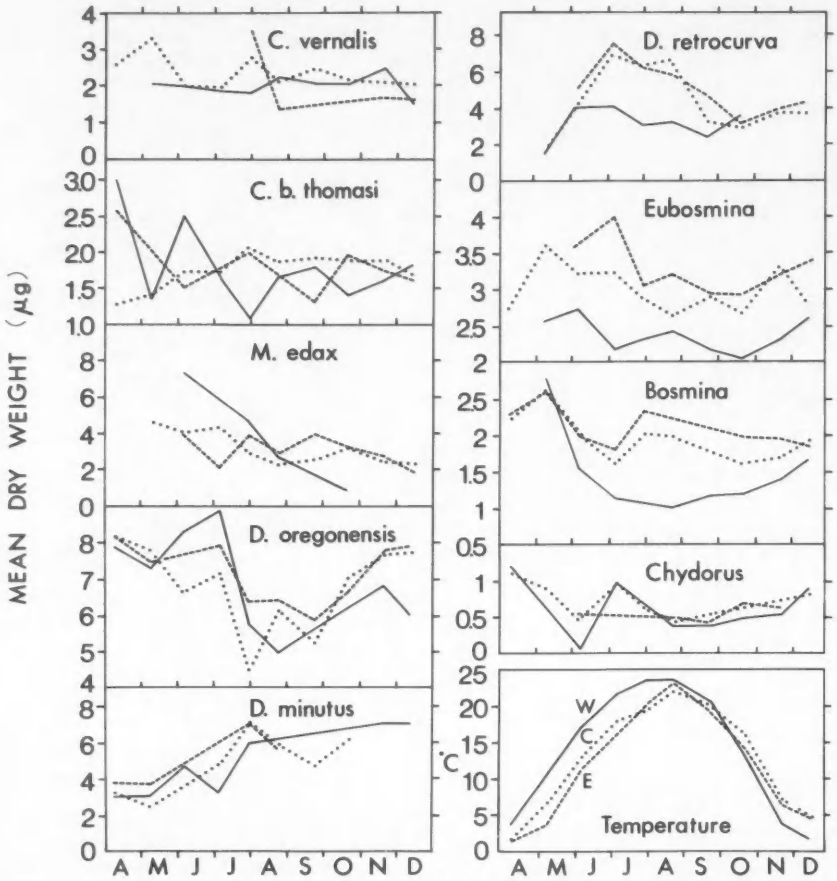

FIG. 4. Seasonal variation in the mean dry weight of major crustacean taxa in lake Erie, 1970. Copepod data include adults only. Cladoceran data include both adult and immature individuals. The variation in average temperature of the water column in each basin is shown in the lower right of the figure.

Dumont et al. (1975), but statistical comparison of slopes and intercepts is not possible from that paper. We have included residual mean square and length variance values in Table 3 so that subsequent regressions can be compared with ours using a *t*-test (Kleinbaum and Kupper 1978). Hawkins and Evans (1979) reported means and ranges of weights without specifying lengths of any of their organisms, so we cannot determine whether length–weight relationships are the same for Lake Michigan populations of our taxa.

Rosen's (1981) study of Connecticut River zooplankton length–weight relationships allows comparison with our results for the seven taxa common to both studies. His methods differed from ours in that he did not rinse the organisms with distilled water, he pipetted them onto small pieces of glass-fiber filters, he weighed them to the nearest microgram, and he divided his specimens among relatively broad (0.04–0.5 mm) size classes. Partly as a result of these differences, his exponent values (b in the equation $W = aL^b$) are consistently higher than ours. Test values of length chosen within ranges for taxa common to both sets of data (*Leptodora kindtii*, *B. longirostris*, *C. sphaericus*, *C. vernalis*, and *M. edax*) generate weights from our equations that are consistently higher than his at the shorter lengths for each taxon, but which converge to similar weights (difference = ±1%) for the top of the range of lengths. The exception to this pattern is *D. retrocurva* for which the two sets of data agree fairly well, and copepod nauplii for which his regressions yield weights that are unreasonably high for the whole range. Rosen (1981) is also an excellent source of length–weight relationships for benthic Cladocera not included in our study.

Comparison of our weights for *L. kindtii* with those of Cummins et al. (1969) disagree by a factor of 10 due to a series of misprints in their table 11 (K. W. Cummins, personal communication). Their (corrected) dry weight of a 2-mm individual, 5.0 μg, agrees well with our value of 6.7 μg for a 2.3-mm individual.

Seasonal Variation in Biomass

Combining the regressions with zooplankton abundance and length–frequency data, we have calculated the biomass of cladocerans and copepods from Lake Erie and cladocerans from Lake Ontario. For Lake Erie, we have converted the basin-wide average biomass estimates in milligrams per cubic metre to areal estimates by multiplying by the average depth of each basin

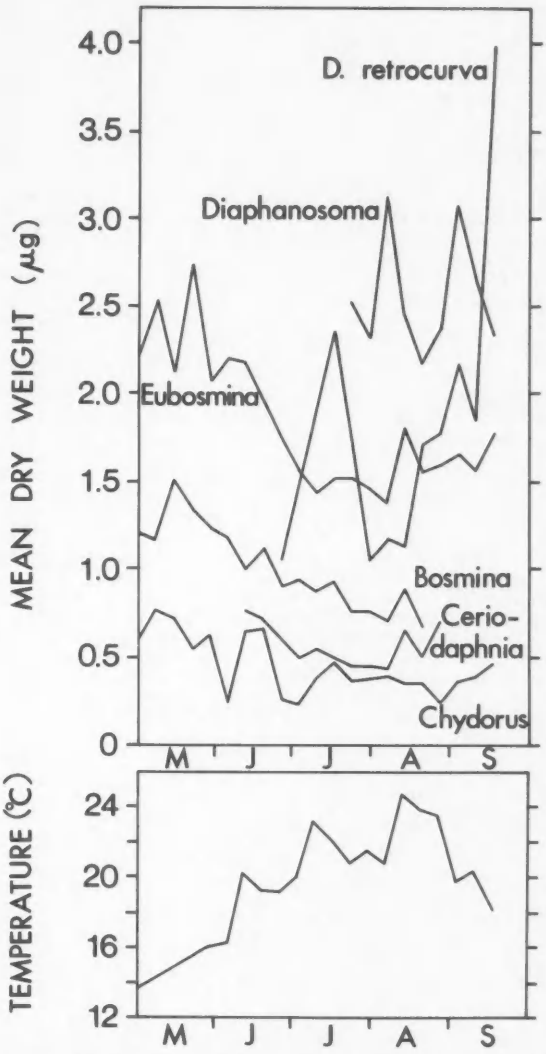

FIG. 5. Seasonal variation in the mean dry weight of major cladoceran taxa from the Bay of Quinte, Lake Ontario, 1974.

(Western = 8 m, Central = 15 m, Eastern = 25 m) (Fig. 3). The three basins show maximal biomass in the summer, with timing and the relative contribution of copepods and cladocerans to the crustacean biomass varying from basin to basin. A similar plot for the Bay of Quinte, Lake Ontario, showed lower midsummer biomass and a greater variability than that seen in Lake Erie. These samples were taken weekly with a Schindler–Patalas trap from a single nearshore station, whereas the multiple-station monthly sampling in Lake Erie masks some of the individual variation present. Watson (1976) assumed 100% filtering efficiency in an unmetered net for his vertical hauls from Lake Erie, so our biomass estimates using his counts from these samples are conservative, but the high standing crop of Lake Erie zooplankton as compared with the Bay of Quinte is obvious. In both lakes the biomass is dominated by cladocerans throughout much of the growing season, with copepods more important early in the year, due primarily to the abundance of *C. b. thomasi*. Size–frequency analyses were not performed on the relatively rare copepods in the Bay of Quinte samples, so copepod biomass was estimated from the individual weights (Table 2).

The seasonal pattern for Lake Erie (Fig. 3) is similar to that found by Watson and Carpenter (1974) for the same samples, but they calculated biomass by multiplying an average individual weight from Wilson and Roff (1973) by abundance for each species. The result was reported as a single lake-wide, volume-weighted average for each cruise, so we cannot compare our results directly. We can, however, determine whether a single estimate for individual weight will give an equivalent biomass estimate to a method that takes body size distribution into account. Wilson and Roff's (1973) average weight for *D. retrocurva* (3.7 μg) is very close to the value we observed during June and July for the Western Basin of Lake Erie (Fig. 4), but it is too low for the larger organisms present at that time of year in the Central and Eastern Basins. Mean dry weight per individual in the Bay of Quinte, Lake Ontario, also varied seasonally (Fig. 5), with some evidence for a negative correlation with temperature change (Culver 1980). These seasonal changes underscore the necessity of taking into account the variations in size which occur seasonally, whatever their ultimate causes. At the same time, our method accounts only for variation in biomass as a result of changes in number and size distribution. Changes in weight per length are not detected.

Accurate biomass estimates of planktonic Crustacea will thus require either direct measurement of the individual weights of taxa in each sample (Hawkins and Evans 1979) or the use of size–frequency determinations and length–weight relationships which are characteristic of the populations under study. Length–weight regressions of organisms collected over a short period of time may not represent the seasonal variation in weight per length that occurs. Some individuals in spring and winter populations of Crustacea are larger than the individuals included in our regressions, but we do not know whether the present equations are accurate for organisms whose lengths fall outside the range used in generating the equations. Changes in the amounts of stored food or in specific gravity occur seasonally (Hutchinson 1967), and we again do not know how they will affect our length–weight relationships. Future work should test their applicability to populations of these species occurring in the other Great Lakes and for organisms collected at other times of the year. Length–weight regressions are also needed for other species that were not common in our samples. We further propose that the ubiquity of many of these species in North America will allow comparisons of length–weight relationships from Lakes Erie and Ontario with other lakes where these species may be found.

Acknowledgements

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Space–Time Correlation and Its Effects on Methods for Detecting Aquatic Ecological Change

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The analysis of variance (ANOVA) is commonly used to analyze observations collected from aquatic monitoring programs designed to detect ecological change. ANOVA assumes that the deviations of the observations from their true means (the errors) are uncorrelated in space and time. Aquatic monitoring data often violate this assumption. The results of Monte Carlo simulations using simulated data generated from both statistically and mechanistically based models show that the presence of either spatially or temporally correlated errors can significantly affect the outcome of ANOVA tests. In practice, spatial correlation is more likely to be a problem than is temporal correlation, given typical monitoring frequencies. The effects of spatial correlation can be minimized through judicious use of control station pairing in the monitoring design. However, when insufficient flexibility exists in the monitoring design, alternate models, such as multivariate time series analysis, or multivariate analysis of variance, must be used in place of ANOVA.

On utilise ordinairement l'analyse de variance (ANOVA) pour analyser les données recueillies dans le cadre de programmes de surveillance du milieu aquatique visant à découvrir des variations écologiques. L'ANOVA suppose que les écarts des données par rapport à leurs vraies moyennes (les erreurs) ne sont pas en corrélation spatiale et temporelle. Les données recueillies au cours d'observations du milieu aquatique réfutent souvent cette hypothèse. Les résultats de simulations Monte Carlo réalisées à l'aide de données fictives produites par des modèles statistiques et mécaniques révèlent que la présence d'erreurs en corrélation spatiale ou temporelle peut nettement influer sur les résultats de tests ANOVA. En pratique, la corrélation spatiale est plus susceptible d'être un problème que la corrélation temporelle étant donné des fréquences typiques d'observations. On peut minimiser l'incidence de la corrélation spatiale en utilisant judicieusement un jumelage des stations de surveillance pour la conception d'un programme. Toutefois, quand cette conception manque de flexibilité, d'autres modèles, dont l'analyse multivariée de séries temporelles ou l'analyse multivariée de la variance, doivent être utilisés au lieu de l'ANOVA.

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Concerns over the possible effects of pollutant discharges have led to legislation in many countries requiring that monitoring programs be implemented to detect possible changes in the aquatic environment. In the United States, much of the required monitoring has been associated with coastal municipal wastewater treatment plants, where a waiver of the secondary treatment requirement is sought, and at electric power generation facilities, particularly those discharging waste heat. In both cases, the objective of the monitoring programs is to determine whether changes in the aquatic environment have occurred that can be attributed to operation of the facility. Demonstration of change may lead to a requirement for additional treatment of waste discharges, or changes in facility management.

Monitoring program expenditures have exceeded $1 million per year at some American power plants; nonetheless, the design of monitoring programs at power plants has been criticized as ineffective (Gore et al. 1979; McKenzie et al. 1977, 1979). In this paper, we address some issues that affect the manner in which the data collected from such project-related aquatic monitoring programs can be analyzed, and how monitoring programs can be designed to avoid the difficulties identified.

One of the most commonly used models for assessing aquatic monitoring data is the analysis of variance (ANOVA). The motivation for ANOVA is that the various factors that might affect the observations can be partitioned and statistical tests conducted to determine whether their effects are distinguishable from the background noise inherent in the data (see, for

example, Sokal and Rohlf 1981, or most other elementary statistics texts).

Models of this type (or their nonparametric equivalents) have been used for assessment of aquatic change by Thomas et al. (1978), Aston and Milner (1980), Mathur et al. (1977), and Evans et al. (1982). The predominant emphasis of these studies on power plant effects reflects the expenditure of resources on environmental monitoring at thermal power plants. In the United States, these monitoring programs are largely in response to Section 301 of the *Federal Clean Water Act* which governs thermal discharges into navigable waters.

As an example of the application of ANOVA, suppose that the density of an organism is to be compared before and after the occurrence of an event that might cause a change in density, say the operation of a new wastewater treatment plant. If observations are taken at various sites in the receiving water before and after the event, a possible model for the data is

$$(1)\quad Y_{jklmn} = \mu + A_j + B_k + C_l + AB_{jk} + AC_{jl} + BC_{kl} + ABC_{jkl} + \eta_{jklm} + \epsilon_{jklmn}$$

where Y_{jklmn} = the observed density for the nth replicate on the mth sampling occasion within season l at station j for event status k, μ = the overall mean density, over stations, event status, and seasons, A_j = main effect of station j (FIXED), B_k = main effect of event status at level k (FIXED), C_l = main effect of season l (FIXED), AB_{jk} = station by event status interaction effect at level jk, η_{jklm} = effect due to sampling occasion m within season l for station j and event status k (RANDOM), ϵ_{jklmn} = random error in the nth replicate on the mth sampling occasion within season l at station j for event status k (RANDOM), $j = 1, \ldots, J$ (station index), $k = 1, 2$ (event status index; 1 = preevent, 2 = postevent), $l = 1, \ldots, L$ (season index), $m = 1, \ldots, M$ (sampling occasion index), and $n = 1, \ldots, N$ (replicate index). Thus, eq. 1 postulates that the observations of density might be affected by the particular station, the season, and whether the event has occurred or not. There are $2M$ sampling occasions per season, assumed for simplicity to be equally apportioned before and after the event. If there is only one sampling occasion per season per year, then M refers to the number of years of sampling for each level of event status. The error term η_{jklm} results from the variability in the observations between sampling occasions at the same station after taking into account the effects of season and event status. The η_{jklm}'s are assumed to be independent random variables distributed normally with mean 0 and variance σ_η^2. The error term ϵ_{jklmn} results from the variability in the observations between replicates taken at the same station and on the same occasion. The ϵ_{jklmn}'s are also assumed to be independent random variables, distributed normally with mean 0 and variance σ_ϵ^2. The zero mean assumption is accommodated in the analysis of variance by the fixed effects. The normality assumption may not be satisfied; however, transformation of the raw data (e.g. logarithmic or power transforms) may make it possible to meet this restriction.

Although it is assumed that the observations at each station reflect the general conditions in the area, it should be noted that the station factor (A) is considered to be a fixed, rather than random, factor in model 1 (eq. 1). The designation of station as a fixed effect is appropriate so long as station locations are chosen in a systematic fashion, for instance, as a result of physiographic characteristics. In some cases, station locations may be selected in a random manner, and the model would have to be changed accordingly.

Using the notation MS($\cdot$) for the computed mean square associated with each factor, the null hypothesis of no change in organism density after occurrence of an event, for instance, H_0: $B_1 = B_2 = 0$, can be tested using the statistic $F = \text{MS}(B)/\text{MS}(\eta)$ which has an F-distribution with 1 and $2JL(M-1)$ degrees of freedom under the null hypothesis. The ANOVA model provides not only a method for analyzing data, but a framework for making monitoring design decisions. For instance, the model suggests that monitoring resources must be allocated to stations (J), seasons (L), sampling occasions (M), and replicates (N). Millard and Lettenmaier (in press) suggest a method for optimizing these allocation decisions.

There is a fundamental problem with applying eq. 1 in practice. Even if the results of the ANOVA indicate a change in organism density after the occurrence of the event, the change can be attributed to the event only if it is known that no other changes occurred in the environment that might also have affected the density. To avoid this problem, control station pairing may be employed. This approach has been advocated by Eberhardt (1976, 1978), McKenzie et al. (1977), and Thomas et al. (1981). If paired control stations are used in the design, then, following Skalski and McKenzie (1982), Y_{jklmn} and A_j in eq. 1 are replaced with

Y_{jklmn} = the observed difference between control and treatment stations for the nth replicate on the mth sampling occasion within season l for station pair j and event status at level k

$$(2)\quad A_j = \text{main effect of station pair } j \text{ (FIXED)}$$

$j = 1, \ldots, J$ (station pair index).

Models similar to eq. 2 have been employed by McKenzie et al. (1977, 1979), Gore et al. (1979), Murarka (1976), Murarka et al. (1976), and Mathur et al. (1980).

The emergence of easy to use statistical software packages has made procedures such as multifactor ANOVA available to most analysts of aquatic monitoring data. Unfortunately, it has also created a trap for the unwary by making it easier to perform statistical tests without understanding or verifying the assumptions upon which the tests are founded. The four basic assumptions of ANOVA are as follows: (1) the deterministic part of the model is additive (in the main effects and interactions), (2) the error terms (η and ϵ) are assumed to be independent (hence uncorrelated), (3) the error terms are homoscedastic (equal variance), and (4) the error terms are normally distributed. These last three assumptions are listed in decreasing order of importance based on the findings of Cochran (1947), Box (1954), and Scheffé (1959) (see also Glass et al. 1972). The independence assumption is usually justifiable for the errors in replicate observations (ϵ), but may be suspect in the case of the errors between sampling occasions (η). Aquatic monitoring data take the form of a multivariate time series because observations at many stations are collected over time. Thus, when an ANOVA model is applied to aquatic monitoring data there are two possible sources of correlation in the η terms: temporal correlation (since the observations are taken sequentially over time) and spatial correlation (since stations close to each other respond similarly to external effects, such as short-term meteorological events and longer term climatic variations). While the deleterious effects of correlated errors on ANOVA have long been recognized by statisticians (e.g. Cochran 1947; Box 1954; Scheffé 1959), the assumptions of spatial and temporal independence are not often varied, or if correlation is present, it is effectively ignored. For instance, Mathur et al.

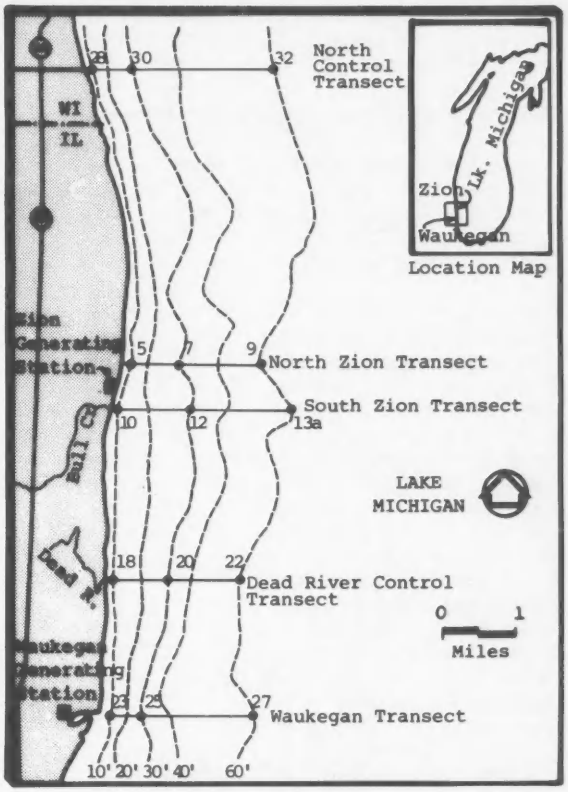

FIG. 1. Zion plankton sampling network (1 mi = 1.6 km).

(1980) found possible evidence of temporally correlated errors in zooplankton densities from Conowingo Pond, but still asserted that the assumptions for the ANOVA tests they performed were satisfied.

In this paper, the effects of temporally and spatially correlated errors on ANOVA procedures that might be used for analyzing aquatic monitoring data are shown, and the possible origin of spatial correlation is demonstrated. The discussion is approached in three sections. The first demonstrates a method for screening aquatic monitoring data for temporal and spatial correlation. The second section describes the results of a Monte Carlo study in which the magnitudes of the temporal and spatial correlations were varied and their effects on the conclusions of ANOVA examined. Finally, the third section demonstrates how spatial correlation can result from a model of phytoplankton biomass with stochastic parameters in the dynamical equation and advection via a turbulent diffusion process.

Exploratory Data Analysis

Before discussing the statistical implications of correlated errors on ANOVA, it is instructive to show the magnitude of the spatial and temporal error correlations in aquatic monitoring data. The aquatic monitoring design at the Zion nuclear power plant (Fig. 1) is typical of similar studies at large thermoelectric power plants. One variable of interest in studies of possible effects of this plant (e.g. Murarka et al. 1976) was the total number of phytoplankton per millilitre. Although other variables were also collected, for illustrative purposes the discussion here is limited to total phytoplankton.

The phytoplankton data reported by Murarka et al. (1976) are monthly samples for the years 1972–75. The data are recorded as one observation per month at each station. It is not clear whether these observations represent the mean of several replicates taken at the same station on the same sampling occasion, or if only one replicate was taken. Since this precludes discrimination of the η and ϵ terms in eq. 1, the spatial and temporal correlations estimated from the model errors will tend to underestimate the true space–time correlation of η (Millard 1985).

The Zion plant began commercial generation on December 31, 1973, but testing began in August 1973 (Murarka et al. 1976). If plant operation is defined to have begun after commencement of commercial operation, then there are 24 observations before plant operation, and 24 after, with stations 28, 30, and 32 missing the first three observations. For the analysis described here, observations at stations 5, 7, 9, 28, 30, and 32 were analyzed. Stations 5, 7, and 9 were considered treatment stations, while stations 28, 30, and 32 were their respective paired control stations. In the context of the models of eq. 1 and 2, for the Zion data $J = 6$ (stations) or 3 (station pairs), $L = 12$, $M = 2$, and $N = 1$. The data for each station were logarithmically transformed prior to analysis, following the recommendation of McKenzie et al. (1977).

In general, for data that can be described by eq. 1 or 2, a test for temporal correlation in the η_{jklm} terms can be performed by deriving the maximum likelihood estimates of autocorrelation and applying a likelihood ratio test (Harvey 1981; Mar et al. 1985). In the case of the Zion data, where η and ϵ cannot be distinguished, ϵ is implicitly set to zero. Then, to test for temporal correlation in the errors (η terms), the time series of estimated errors $\hat{\eta}_1, \ldots, \hat{\eta}_T$, where $T = 2LM$ (the total number of sampling occasions), must be formed for each station (pair). Equation 1 or 2 is used to compute the $\hat{\eta}_t$'s once the fixed effects in the ANOVA model have been estimated. Explicitly,

$$\hat{\eta}_{jklm} = Y_{jklm} - \bar{Y}_{jkl}, \text{ where } \bar{Y}_{jkl} = (1/M) \sum_{m=1}^{M} Y_{jklm}.$$

The simple lag-one correlation ϕ is estimated by

$$(3) \quad \hat{\phi} = \frac{\sum_{t=1}^{T-1} \hat{\eta}_t \hat{\eta}_{t+1}}{\sum_{t=1}^{T} \hat{\eta}_t^2}$$

and is approximately normally distributed with mean 0 and variance $1/T$ if the (population) value of ϕ is zero (Box and Jenkins 1976, p. 34). For the Zion phytoplankton data, temporal correlation in the errors at each station was negligible; none of the estimated correlations was significantly different from 0 (two-sided p values ranged from 0.16 to 0.36).

As in the case of temporal correlation, the estimation of spatial correlation in the η_{jklm} terms is complex when the ϵ_{jklmn} terms are included in the model (Mar et al. 1985), but the effect of excluding the ϵ terms is to bias the spatial correlation downward so long as the ϵ's are independent. The correlation between station (pair) j and j' is estimated by

$$(4) \quad \hat{\rho}_{jj'} = \frac{\hat{\sigma}_{jj'}}{[\hat{\sigma}_{jj}\hat{\sigma}_{j'j'}]^{1/2}}$$

TABLE 1. Estimates of cross-correlation between the north stations at Zion. Underlined values are correlations between treatment–control pairs.

	Station				
Station	5	7	9	28	30
7	0.65				
9	0.75	0.83			
28	<u>0.79</u>	0.47	0.79		
30	0.62	<u>0.67</u>	0.87	0.85	
32	0.56	0.51	<u>0.77</u>	0.77	0.89

TABLE 2. Estimates of cross-correlation in the treatment–control pair differences at Zion.

	Station pair	
Station pair	5–28	7–30
7–30	0.86	
9–32	0.54	0.69

where

$$\hat{\sigma}_{jj'} = \sum_{k=1}^{2} \sum_{l=1}^{L} \sum_{m=1}^{M} (\hat{\eta}_{jklm}\hat{\eta}_{j'klm})/[2L(M-1)].$$

If R denotes the $J \times J$ matrix of estimated correlation coefficients, then the statistic

$$(5) \quad \chi^2 = -\left[T - 1 - \frac{(2J+5)}{6}\right] \ln |R|$$

is approximately distributed as a chi-square random variable with $J(J-1)/2$ degrees of freedom under the null hypothesis that spatial correlation is absent (Morrison 1976, p. 118). For the observations at single stations at Zion, spatial correlation in the errors (between stations) is clearly present, as shown in Table 1. The computed χ^2 of 326.3 associated with 15 degrees of freedom yields a p value less than 0.001. The autocorrelation in the treatment–control pair differences, on the other hand, was not significantly different from zero for pairs 7–30 and 9–32 ($p = 0.2$ and $p = 0.9$), although it was significant for pair 5–28 ($p = 0.04$). Table 2 shows the cross-correlations for the treatment–control pair differences. Tables 1 and 2 show that high spatial correlation exists between the errors in the differences, as well as the raw station data. The likelihood ratio tests indicate that all cross-correlations are significantly different from zero at the $\alpha = 0.001$ level.

Although the results presented here are specific to the Zion site, Mar et al. (1985) have conducted similar analyses at several sites for phytoplankton, zooplankton, and benthic data. In all cases, temporal correlation was found to be negligible whereas in most cases, spatial correlation was high. This result is, of course, conditioned on the sampling interval, which was usually 1 mo for the data analyzed; higher temporal correlation should be present at shorter sampling intervals. In general, though, temporal correlation is not likely to be as significant a concern in application of ANOVA to aquatic monitoring data as is spatial correlation. In the remainder of this paper, we assess the effect of spatial correlation on ANOVA-based tests for aquatic change using Monte Carlo simulation methods, and subsequently we demonstrate the physical/biological basis for spatial correlation in phytoplankton observations.

Monte Carlo Study — Statistical Simulation

Given the magnitude of the spatial correlation present in the Zion phytoplankton data and in other aquatic variables analyzed by Mar et al. (1985), it is important that the effect of spatial correlation on a paired treatment–control ANOVA be assessed. Earlier work by Cochran (1947), who studied the effects of correlated errors on a one-way ANOVA, and Box (1954), who studied the effects of correlated errors on a two-way ANOVA with one observation per cell, provides some basis for such an assessment. However, both these studies made certain simplifying assumptions about the nature of the correlations, and so are somewhat limited. For the purposes of this study, it was desired to quantify the effects of correlated errors on ANOVA procedures that employ models similar to those used in aquatic ecological monitoring, and when the spatial correlation assumed certain parametric forms. Therefore, a Monte Carlo simulation procedure, which requires no simplifying assumptions, was used. Although it appears that temporal correlation is likely to be a less serious problem, the simulation model retains the option for generating temporally correlated data as well.

The purpose of the Monte Carlo study described here was to quantify the effects of spatially and temporally correlated errors on the type I error (the probability of rejecting the null hypothesis when in fact it is true) for ANOVA models similar to those given in eq. 1 and 2. The station layout used is shown in Fig. 2 and is similar to the station layouts used at the Zion (Murarka et al. 1976), Nine Mile Point (Murarka 1976), and Cook (Evans et al. 1982) power plants. Observations were generated at each sampling station with errors correlated in space and time, using a normal random number generator. The synthetic data generator assumed the same variance and temporal correlation at each station. Therefore, the error process was separable, that is, the spatial correlation and the temporal correlation could be factored. The temporal correlation was taken to be a lag-one autoregressive process (exponentially decaying correlation in time), while the spatial correlation was taken to be an isotropic, exponentially decaying function. Therefore, the space–time correlation of the errors was of the form

$$(6) \quad C(S(\boldsymbol{x}_i, t+\tau), S(\boldsymbol{x}_j, t)) = \phi^{|\tau|} \exp(-\kappa h_{ij})$$

where $C(\cdot)$ = space–time correlation function, $S(\boldsymbol{x}_i, t)$ = observation at station i at time t, $\boldsymbol{x}_i$ = position vector of station i, $h_{ij} = |\boldsymbol{x}_i - \boldsymbol{x}_j|$, the distance between station i and j, ϕ = temporal correlation parameter, $0 \leq \phi < 1$, and κ = spatial correlation parameter, $0 < \kappa < \infty$. Note that the spatial correlation between two stations decreases as κ increases. The assumption of isotropy is open to question due to the influence of such factors as depth and current, which could lead to an anisotropic correlation function. The correlation model given in eq. 6 was favored, however, because it is simple but retains the essential elements of a spatially and temporally correlated random process.

The model used to simulate observations is given by eq. 1 with $J = 6$ stations, $L = 12$ seasons, $M = 2$ yr of sampling for each level of event status, and $N = 1$ replicate. The ϵ_{jklmn} term was set to zero, so that the results would isolate the effects of temporal and spatial correlation in the η terms.

TABLE 3. Hypotheses tested in the Monte Carlo studies.

H_0:	$ABC_{jkl} = 0$	for all j, k, l	(no Station Pair × Event Status × Season effect)
H_1:	$AB_{jk} = 0$	for all j, k	(no Station Pair × Event Status effect)
H_2:	$BC_{kl} = 0$	for all k, l	(no Event Status × Season effect)
H_3:	$B_k = 0$	for all k	(no Event Status effect)

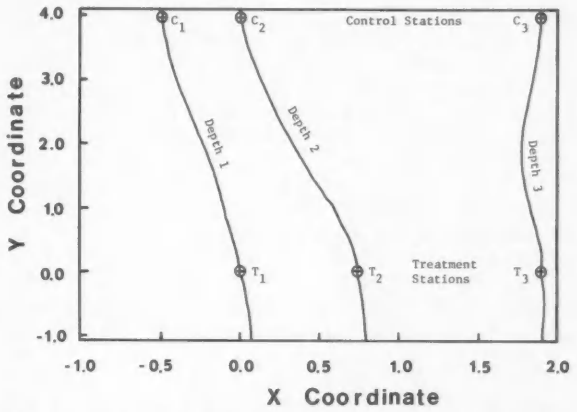

FIG. 2. Sampling network for Monte Carlo statistical simulation study.

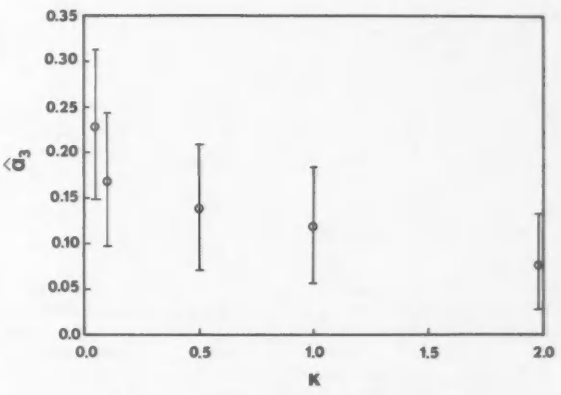

FIG. 3. Event Status type I error vs. κ.

The data generator allows the error term η_{jklm} to be correlated in space or time. Similarly, since the objective of the study was to determine the effects of correlation in the error term η_{jklm}, all other terms in eq. 1 were set to zero when the simulated data were generated (i.e. the null hypotheses H_0–H_3 were true). Once the data for each station were generated, station pair differences were formed (Fig. 2) and the model of eq. 2 fit to these differences. Hence, for the pair differences data, $J = 3$ station pairs.

Table 3 shows the hypotheses tested. The interest in H_0, H_1, and H_2 is due to the complications introduced by nonzero interaction terms in performing the test of H_3: no Event Status effect (Scheffé 1959). It is common practice to use a conditional testing procedure to test hypotheses related to an ANOVA model. If a test for interaction indicates no significant effect, then the tests for the main effects comprising the interaction term are carried out. If, however, an interaction effect is significant, then the tests for main effects are usually not performed. For example, a rejection of H_2 would imply a differential event impact across seasons, and the data for each season would be examined separately to determine the nature of impact. Similarly, a rejection of H_1 would imply a differential plant impact across station pairs (depth contours, Fig. 2). On the other hand, because all effects are absent in this Monte Carlo study, one could also carry out an unconditional testing procedure.

In the Monte Carlo study, 100 trials were carried out for each set of parameters that determined the specific magnitudes of spatial and temporal correlation in the errors. In all tests, the reference value for α (type I error probability in the absence of spatial or temporal correlation) was 0.05. Each trial consisted of the following:

(1) The generation of 48 observations at each station (24 observations in the preevent phase, and 24 in the postevent phase). The standard deviation of the observations was set to 0.6 (recall that the simulations are carried out in log-transform space) based on review of several sets of monitoring data for phytoplankton (Mar et al. 1985). Sensitivity to a much larger standard deviation (50) was also tested in some cases.

(2) The calculation of the differences between the treatment and control stations. All differences were distributed as normal random variables with mean 0. Thus, the hypotheses H_0–H_3 were always true.

(3) The application of the F-tests of H_0–H_3 (Scheffé 1959). Both unconditional and conditional tests were carried out. Under the conditional testing criteria, H_1 and H_2 were not tested if H_0 was rejected, and H_3 was not tested if H_0, H_1, or H_2 was rejected. In all the trials, the F-statistic used to test a hypothesis was compared with the 95th percentile of its true distribution under the null hypothesis (in the absence of any correlation in the errors). Thus, in the absence of temporal and spatial correlation, H_0–H_3 should have been rejected about 5% of the time.

The results of the Monte Carlo study are shown in Tables 4 and 5 and Fig. 3 and 4. Figures 3 and 4 show the estimated type I error associated with testing H_3, along with the large sample 95% confidence limits for the true value of the type I error. If α_i is the probability of rejecting H_i when in fact H_i is true ($i = 0, 1, 2, 3$; see Table 3), and $\hat{\alpha}_i$ is the estimate of α_i based on the results of r Monte Carlo trials, then the 95% confidence limits for α_i are

$$(7) \qquad \hat{\alpha}_i \pm 1.96[\hat{\alpha}_i(1 - \hat{\alpha}_i)/r]^{1/2}$$

where r is the number of times the hypothesis H_i was tested. Figures 3 and 4 show the results for the unconditional testing procedure; hence, for these figures $r = 100$. Note that the magnitude of the second term in eq. 7 (hence the width of the confidence bands in Fig. 3 and 4) varies as $r^{-1/2}$; therefore, $\hat{\alpha}_i$ approaches its population value if r is made large enough. However, the general character of α_i is apparent for $r = 100$.

The Monte Carlo results show that increasing spatial correlation (Table 4) increases the type I error probability with respect to the tests for Event Status, and Event Status × Season effects.

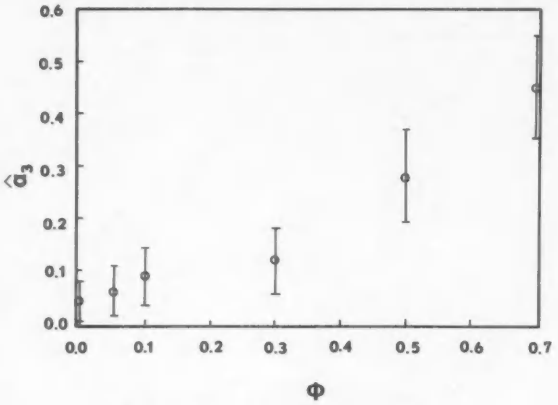

FIG. 4. Event Status type I error vs. ϕ.

TABLE 4. Results of the Monte Carlo statistical simulation study: effects of spatial correlation using unconditional tests (conditional test results in parentheses). ϕ = the lag-one correlation at each station, κ = the parameter of the spatial correlation function (see eq. 6), and $\hat{\alpha}_i$ = the estimate of the probability of rejecting H_i (see Table 3) when in fact it is true.

ϕ	κ	$\hat{\alpha}_0$	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$
0	5.00	0.04	0.01 (0.01)	0.04 (0.03)	0.04 (0.03)
0	2.00	0.01	0.02 (0.01)	0.08 (0.07)	0.08 (0.09)
0	1.00	0.00	0.02 (0.02)	0.29 (0.29)	0.12 (0.12)
0	0.50	0.00	0.01 (0.01)	0.48 (0.48)	0.14 (0.13)
0	0.10	0.00	0.00 (0)	0.66 (0.66)	0.17 (0.12)
0	0.05	0.00	0.00 (0)	0.65 (0.65)	0.23 (0.23)

TABLE 5. Results of the Monte Carlo statistical simulation study: effects of temporal correlation using unconditional tests (conditional test results in parentheses).

ϕ	κ	$\hat{\alpha}_0$	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$
0.00	5	0.04	0.01 (0.01)	0.04 (0.03)	0.04 (0.03)
0.05	5	0.07	0.05 (0.05)	0.07 (0.06)	0.06 (0.06)
0.10	5	0.04	0.05 (0.05)	0.05 (0.05)	0.09 (0.09)
0.30	5	0.02	0.15 (0.15)	0.03 (0.03)	0.12 (0.11)
0.50	5	0.03	0.33 (0.32)	0.04 (0.04)	0.28 (0.29)
0.70	5	0.02	0.47 (0.47)	0 (0)	0.45 (0.50)

Increasing temporal correlation (Table 5) increases the type I error probability with respect to the tests for Event Status × Station Pair and Event Status effects. Interestingly, increasing spatial correlation appears to decrease the type I error for the test of the three-way interaction effect.

It has already been noted that a significant Event Status × Season effect indicates the existence of a differential event effect across seasons, and a significant Event Status × Station Pair effect indicates the existence of a differential effect across depth contours. If none of the interaction terms involving factor *B* (Event Status) are significant, then a significant Event Status effect indicates the existence of an impact that is equal in magnitude across all seasons and depths. The results cited above show that for a conventional ANOVA procedure applied to aquatic monitoring data, the actual type I error probability for a test of an event effect can be several times the nominal value when the errors are highly correlated in space or time. Alternatively, if the type I error probability is somehow maintained at the nominal level, the power will be decreased compared with the power associated with methods that explicitly account for correlated errors. The indication of a significant impact will appear either as an interaction effect (Event Status × Station Pair or Event Status × Season), or as a main effect (Event Status).

The results for the conditional and unconditional tests were quite similar because all the hypotheses H_0–H_3 were in fact true for the generated data. Also, the results for the two different values of the standard deviation of the observations were similar. Under the null hypothesis, the *F*-statistic is a ratio of two different estimates of the same variance; hence, it is to be expected that the results will be approximately invariant with respect to the magnitude of the true variance.

The results for the test of no Event Status effect (H_3) in the presence of increasing temporal correlation (Table 5) are in accord with the findings of Cochran (1947) and Box (1954). They showed that positive correlation within groups increases the type I error probability when comparing means between groups. For each station pair, the presence of temporal correlation at the single stations can be shown to induce serial correlation in the station pair differences (Millard 1985), and hence within each level of Event Status. Thus, positive temporal correlation increases the type I error probability for a test of Event Status effect.

The results for testing for no Event Status effect in the presence of increasing spatial correlation (Table 4) also agree with the findings of Cochran (1947) and Box (1954). The presence of positive spatial correlation among stations induces positive correlation among the station pair differences for each sampling occasion (Millard 1985). Hence, positive spatial correlation induces positive correlation within each level of Event Status, so the type I error probability for testing for this effect is inflated.

The above findings show that the application of conventional ANOVA procedures to aquatic monitoring data that contain spatially or temporally correlated errors will result in a higher probability of declaring that an impact has occurred when in fact it has not. Although for most aquatic monitoring data with sampling intervals on the order of a month, temporal correlation is negligible, spatially correlated errors are often present and will inflate the probability of a type I error. Therefore, it is important, if ANOVA is to be applied, that the error independence assumption be carefully checked. In many cases, the independence assumption will not hold, particularly with respect to spatial correlation. In the following section, for instance, the physical/biological basis for the presence of spatially correlated errors in phytoplankton data is demonstrated.

A Physical Basis for Correlation

Based on the Zion data, and additional analyses reported by Mar et al. (1985), it appears that phytoplankton abundance will have small temporal correlation when the sampling interval is on the order of a month or longer, but will be highly spatially correlated even when station separation distances are on the order of several miles. A possible explanation for the lack of temporal correlation in phytoplankton is the high turnover rate of phytoplankton relative to the length of the sampling interval, since typical generation times are on the order of a week (Wetzel

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

FIG. 5. Hypothetical lake used for Monte Carlo phytoplankton simulation study.

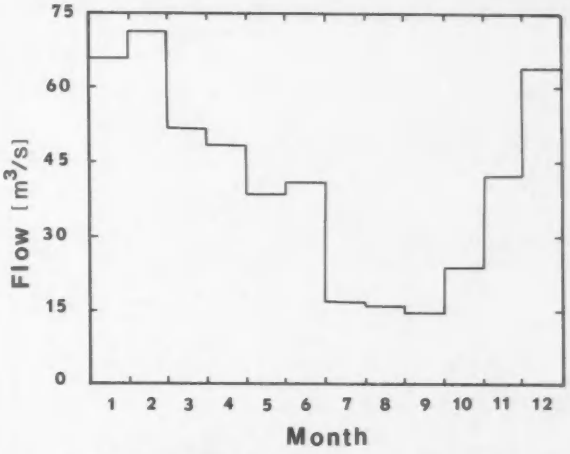

FIG. 6. Freshwater discharge to hypothetical lake used for Monte Carlo phytoplankton simulation study.

1983). The presence of spatial correlation, on the other hand, might be caused by the same phenomena thought to cause phytoplankton patchiness: population blooms coupled with turbulent diffusion (Fasham 1978). If, for some reason, there is a sudden increase in the phytoplankton population in a particular area, turbulent diffusion can shift part of this bloom into contiguous areas. If the sampling interval is long compared with the length of time it takes for a bloom to diffuse, and if some sampling stations are close enough to each other that the effects of diffusion between them are large relative to natural variability over time, then the data from such a sampling program will have spatially correlated errors for the stations that are close to each other. The effect of turbulent diffusion, then, should be to cause observations from sampling stations that are close together to deviate from their means in the same direction.

To further investigate this mechanism, and to determine whether it is consistent with the observed correlation in phytoplankton data, ANOVA procedures were applied to data generated by a mathematical model of phytoplankton population dynamics in a hypothetical 100 square unit (10 × 10) lake (Fig. 5). The model incorporates the process of turbulent diffusion, and hence there was a physical basis for the possible presence of spatial correlation in the data generated by this model.

The unit of measure for the lake was taken to be 914 m (3000 ft); thus, the lake covered an area of 84 km^2 (20 661 acres). The lake was assumed to be uniformly 6 m (20 ft) in depth; hence, it contained 5.1×10^8 m^3 (1.8×10^{10} ft^3) of water. Each cubic unit of the lake (a volume of 5.1×10^6 m^3) received the same amount of phosphorus input via a freshwater inflow. The inflow of water varied monthly (Fig. 6).

The model to describe the phytoplankton dynamics uses Michaelis–Menton kinetics and is structurally similar to other phytoplankton models (Chen and Orlob 1975; DiToro and Matystik 1980). The mechanics of the model are described below.

If grazing by zooplankton can be ignored (e.g. Gasperino and Soltero 1977), the dynamics of phytoplankton primary production can be described (Water Resources Engineers, Inc. 1975) by

$$(8)\quad \partial M(x, t)/\partial t + V(t)\cdot\vec{\nabla}M(x, t) = \vec{\nabla}D\cdot\vec{\nabla}M(x, t) + [\lambda(x, t) - \gamma - \theta]M(x, t)$$

where M = the algal biomass (milligrams carbon per litre), V = the velocity vector, D = the diffusion coefficient, λ = the algal growth rate, γ = the algal respiration rate, θ = the algal sinking rate, x = the 3 × 1 vector of coordinates of a point in the lake, and t = time. For this study, the algal growth rate, λ, was described by

$$(9)\quad \lambda(x, t) = \mu\left(\frac{P(x, t)}{K_P + P(x, t)}\right)\left(\frac{Q(t)}{K_Q + Q(t)}\right) + \tilde{\mu}(x, t)$$

where μ = maximum specific growth rate, $\tilde{\mu}$ = random variation in the growth rate distributed as $N(0, \sigma_\mu^2)$, P = concentration of inorganic phosphorus, K_P = half-saturation constant for phosphorus, Q = photosynthetically active portion of solar radiation, and K_Q = half-saturation constant for light. The concentration of inorganic phosphorus, P, was described by

$$(10)\quad \partial P(x, t)/\partial t + V(t)\cdot\vec{\nabla}P(x, t) = \vec{\nabla}D\cdot\vec{\nabla}P(x, t) + (\omega - \beta\lambda)P(x, t)$$

where ω = rate at which respired algal biomass is converted to inorganic phosphorus and β = ratio of phosphorus to total biomass in the algal population.

Equations 8–10 were solved numerically using a finite difference scheme for a two-dimensional (vertically well-mixed) body of water. The phosphorus concentration of the inflow was maintained at a constant value of 0.05 mg/L throughout the simulation. The photosynthetically active portion of solar radiation (Q) was determined from daily-averaged values of observed solar radiation measured at Seattle–Tacoma International Airport during the period 1952–71 (Fig. 7). The coefficient of horizontal eddy diffusion (D) was assumed to be 0.93 m^2/s (10 ft^2/s), which is very near to the upper limit for numerical stability in the finite difference scheme given the dimensions of the lake; however, it lies within the range of values recommended by Mancini et al. (1981). The other

TABLE 6. Parameter values used for the phytoplankton simulation model.

Parameter	Description	Value
D	Diffusion coefficient	0.93 m^2/s
γ	Algal respiration rate	0.04 $days^{-1}$
θ	Algal sinking rate	0.06 $days^{-1}$
μ	Maximum specific growth rate	0.2 $days^{-1}$
σ_μ	Standard deviation of growth rate noise term	0.2 $days^{-1}$
K_P	Half-saturation constant for phosphorus	0.01 mg/L
K_Q	Half-saturation constant for light	0.01 $kcal \cdot m^{-2} \cdot s^{-1}$
ω	Rate of conversion of biomass to phosphorus	0.05 $days^{-1}$
β	Ratio of phosphorus to biomass	0.02

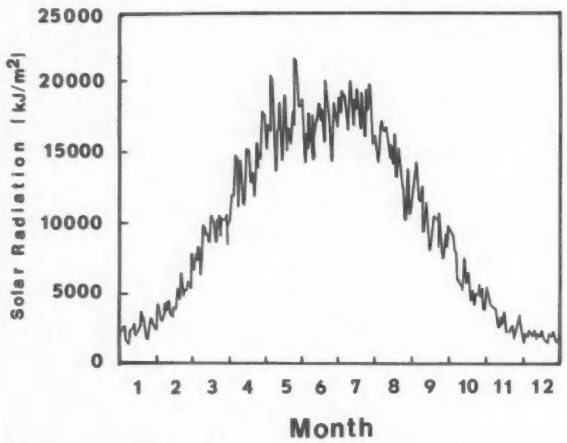

FIG. 7. Average daily solar radiation at Seattle–Tacoma International Airport for the period 1952–71.

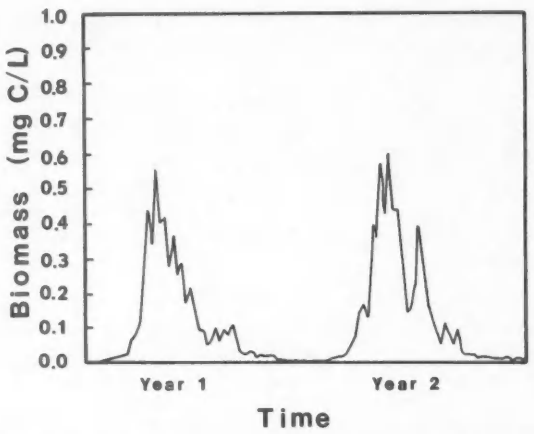

FIG. 8. Typical 2-yr sequence of algal biomass generated by the simulation model.

parameters required for the simulation are given in Table 6 and are typical of those in the literature (Zison et al. 1978). The only stochastic variable in the simulation was the phytoplankton growth rate (λ) as described by eq. 9. This formulation is similar to the one used by May (1973) in his anlysis of marginal distributions associated with the logistic equation. Examples of Monte Carlo simulation studies of lake eutrophication in which growth was assumed to be a random variable are Scavia et al. (1981) and Tiwari and Hobbie (1976). The standard deviation of the random component (σ_μ) in this study was 0.2 $days^{-1}$.

The simple phytoplankton model described by eq. 8–10 was used to generate daily-averaged populations of algal biomass (milligrams carbon per litre) in the hypothetical lake for fifty 4-yr sequences. A typical 2-yr sequence is shown in Fig. 8. Five treatment–control station pairs at various separation distances were hypothesized. The various configurations are given in Table 7. Adjacent treatment stations were always 914 m apart, as were adjacent control stations, but the distance between a treatment station and its paired control station was allowed to vary from 914 to 4572 m (3000 to 15000 ft). The model output was observed at each station every 28 d, beginning at day 5, throughout the year for a total of 13 observations per year at each station. The year was divided into 13 seasons, so at each station there was one observation per season per year. The ANOVA model of eq. 2 was used to model the station pair differences in log (algal biomass). The first 2 yr of data were taken to be preoperational years of a hypothetical power plant located by the lake, and the third and fourth years were operational years. Thus, for the ANOVA model there were $J = 5$ station pairs, $L = 13$ seasons, $M = 2$ yr of sampling for each level of plant status, and $N = 1$ replicate. The ϵ term of eq. 2 was ignored because $N = 1$ and because there was, in fact, no replicate error (each generated observation was the true population value). For each of the 50 sequences, hypotheses H_0–H_3 (Table 3) were tested at the 0.05 level (probability of a type 1 error) under the conditional testing criteria. As for the Monte Carlo experiments reported in the previous section, hypotheses H_0–H_3 were known to be true for the data simulated by this phytoplankton model.

The results of the phytoplankton simulation Monte Carlo study are shown in Table 8. These results are qualitatively similar to the results for the Monte Carlo statistical simulation study when spatial correlation was present and temporal correlation absent (Table 4). The type I error for the test of the three-way interaction effect appears well below the assumed 0.05 level. The type I error for the test of Plant Status × Season effect is above the 0.05 level and increases with increasing separation distance between treatment–control pairs. The type I error for the test of Plant Status effect, however, does not appear to be inflated, except perhaps for the largest separation distance.

A value of 0.2 $days^{-1}$ for the maximum specific growth rate μ (Table 6) implies that the generation time of the phytoplankton is on the order of 5 d. Hence, the sampling interval of 28 d is much longer than the generation time, so, based on the mechanism suggested earlier, it is expected that temporal

TABLE 7. Sampling configurations used in the phytoplankton simulation model Monte Carlo study (see Fig. 5).

Separation distance (m)	Treatment–control pair				
	1	2	3	4	5
914	21–22	31–32	41–42	51–52	61–62
1829	21–23	31–33	41–43	51–53	61–63
2743	21–24	31–34	41–44	51–54	61–64
4572	21–26	31–36	41–46	51–56	61–66

TABLE 8. Results of the Monte Carlo phytoplankton simulation study using conditional tests (number of trials for each $\hat{\alpha}_i$ in parentheses).

Separation distance (m)	$\hat{\alpha}_0$	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$
914	0.00 (50)	0.02 (50)	0.12 (50)	0.07 (43)
1829	0.00 (50)	0.06 (50)	0.30 (50)	0.03 (33)
2743	0.00 (50)	0.08 (50)	0.34 (50)	0.06 (33)
4572	0.00 (50)	0.04 (50)	0.46 (50)	0.19 (27)

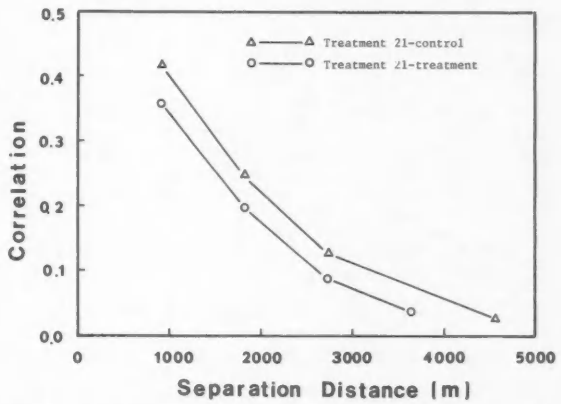

FIG. 9. Correlation between treatment station 21 and paired controls, and between station 21 and the other treatment stations (31, 41, 51, and 61).

correlation of the generated data is small. On the other hand, a value of 0.93 m^2/s for the coefficient of horizontal diffusion, D (Table 6), implies that it takes about 10 d for an area the size of one unit of the lake (8.4×10^5 m^2) to mix. Thus, if a sampling occasion occurs up to about 10 d after a bloom has occurred at a station, spatially correlated errors between that station and its neighboring stations may be evident.

The estimated auto- and cross-correlations from the 200 yr of simulated data showed that temporal correlation is essentially absent, but spatial correlation is present and decays with distance (Fig. 9). The correlations associated with the errors were estimated using the residuals (observations that had been standardized by subtracting the seasonal mean). The estimates of lag-one autocorrelation at each of the 25 hypothesized stations used in the Monte Carlo study ranged from 0.03 to 0.11, with an average of 0.06. These estimates are based upon $T = (2)(L)(M)(50) = (2)(13)(2)(50) = 2600$ observations.

Figure 9 shows the estimated spatial correlation between treatment station 21 and its paired control station for the four separation distances considered (Table 7), and between station 21 and the other treatment stations (31, 41, 51, and 61; see Fig. 5). The standard deviations of these estimates is on the order of $2600^{-1/2} = 0.02$; hence, 95% confidence limits are not shown. Figure 9 shows that spatial correlation is large between adjacent stations, decays with distance, and that for large separation distances between treatment–control pairs the treatment stations are more highly correlated with each other than with their paired control stations. Plots using other treatment stations as the reference station gave similar results.

Conclusions

In the process of creating a sampling design to monitor the aquatic environment for ecological change, the consequences of the two kinds of false conclusions that might be drawn should be compared. If a type I error occurs (i.e. it is concluded that there has been a change when in fact no change has occurred), unnecessary expenditure for environmental controls may be incurred. If a type II error occurs (i.e. it is concluded that there has been no change in the environment when in fact a change has occurred), direct and indirect costs due to unabated environmental damage may result. The standard statistical procedure to control both types of errors is to first set the probability of a type I error at a known level and then to either calculate the sample size required to keep the type II error below a set probability for a given magnitude of change (minimum detectable difference) or calculate the probability of a type II error for a given magnitude of change when the sample size (the cost of the program) has been fixed (e.g. Snedecor and Cochran 1980, p. 102–105). In both cases, however, it is assumed that the type I error probability has been fixed a priori. The results of this work show that independence of the errors in ANOVA models is a critical assumption affecting type I error, and often will not be justifiable for aquatic monitoring data.

Specifically, the following has been shown:

(1) The type I error for tests of environmental change due to an intervention (e.g. power plant start up) is likely to be inflated if the observations are positively correlated in time or space.

(2) Spatial correlation will usually be stronger than temporal correlation for typical sampling frequencies, for instance, bimonthly to monthly.

(3) Spatial correlation is of greatest concern when the errors at the control (treatment) stations are more highly correlated than the errors between the treatment–control pairs.

(4) Spatial and temporal correlations observed in phytoplankton data are consistent with the space–time scales of the parameters of a turbulent diffusion model.

The question remains of how aquatic monitoring data should be analyzed when spatial or temporal correlation is present in the errors. One possible way in which the problem can be minimized is to separate the control (treatment) stations by large distances relative to the distance between treatment–control pairs. This is sometimes infeasible because of the relatively small size of the potentially affected area. Another approach is to use alternate statistical techniques to plan the monitoring design and analyze the data. For example, if temporal correlation is present, multivariate time series analysis (e.g. Harvey 1981) may be used. If only spatial correlation is present, then multivariate analysis of variance (Johnson and Wichern 1982) is appropriate. These models are discussed in Mar et al. (1985). In any event, it is important that the absence of spatial and tem-

poral correlation in the errors be verified if ANOVA tests are to be applied.

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Historical Relationships between Phosphorus Loading and Biogenic Silica Accumulation in Bay of Quinte Sediments[1]

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Schelske, C. L., D. J. Conley, and W. F. Warwick. 1985. Historical relationships between phosphorus loading and biogenic silica accumulation in Bay of Quinte sediments. Can. J. Fish. Aquat. Sci. 42: 1401–1409.

Sediments from Warwick's 1972 Glenora-B core from the Bay of Quinte, Lake Ontario, were analyzed to compare the historical relationship between the accumulation of biogenic silica (BSi) and total phosphorus (TP). The similarities in patterns provide evidence that increased phosphorus (P) inputs caused increased diatom production and BSi accumulation. BSi accumulation increased soon after initial European settlement by French Sulpicians in 1669 and reached maximum levels during the early 1850s when forest clearance and erosion of the deforested drainage basin increased sediment accumulation rates 110-fold compared with rates before 1669. Maximum rates of BSi and TP accumulation increased 170-fold and 150-fold, respectively, during the same period. Ratios of BSi:TP were about sixfold greater in the sediments deposited after 1888 than in those deposited prior to 1669, indicating that the proportion of available P in TP inputs increased with increased disposal of domestic sewage into the bay as populations shifted to urban centers. Increases in BSi accumulation above the *Ambrosia* horizon (dated at 1852) indicate that sediments can be a significant sink for BSi. Although the onset of Si depletion cannot be confirmed with data from this core, there is clear evidence that BSi accumulation increased as the result of increased P inputs.

Une carotte (Glenora-B) prélevée en 1972 par Warwick dans la baie de Quinte (lac Ontario) a été analysée afin de comparer, sur une grande période, les rapports d'accumulation de la silice biogène (SiB) et du phosphore total (PT). La similitude des tendances indique que l'augmentation des apports de phosphore (P) s'est traduite par un accroissement de la production de diatomées et de l'accumulation de la SiB. Cette dernière a augmenté peu de temps après l'arrivée des premiers Européens, des Sulpiciens français en 1668, et a atteint un maximum au début des années 1850, quand la déforestation et l'érosion du bassin hydrographique dénudé ont provoqué une augmentation des taux de sédimentation qui ont été multipliés par 110 par rapport à ceux d'avant 1669. Au cours de la même période, les taux maximums d'accumulation de SiB et de PT ont augmenté respectivement de 170 et de 150 fois. Le rapport SiB/PT était environ six fois plus grand dans les sédiments déposés après 1888 que dans ceux déposés avant 1669. Cela indique que la proportion de P disponible des apports de PT s'est accrue avec l'augmentation du rejet d'eaux usées domestiques dans la baie à mesure que la population se concentrait dans les centres urbains. La plus grande accumulation de SiB dans les sédiments supérieurs à l'horizon *Ambrosia* (1852) indique que ces derniers peuvent constituer un réservoir important pour le SiB. Les données obtenues à partir de cette carotte ne permettent pas de confirmer le début de l'appauvrissement en silice, mais il apparaît clairement que le taux d'accumulation du SiB a augmenté suite à l'augmentation des apports de P.

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Several previous studies related increased phosphorus (P) loading to increased silica (Si) utilization by diatoms in the Laurentian Great Lakes. Schelske and Stoermer (1971, 1972) hypothesized that the imbalance between P and Si inputs to Lake Michigan increased diatom production and resulted in epilimnetic Si depletion and Si-limited diatom growth in the late 1960s. Dobson et al. (1974) related the rate of Si uptake by diatoms in the trophogenic zone to total P concentration, and Schelske (1975) showed that areal Si depletion could be used to assess the degree of eutrophication in the upper Great Lakes. Although the Si depletion hypothesis has been questioned from previous studies of sediments, recent data from sediment cores show that Si depletion occurred not only in Lake Michigan in the late 1960s, but also in Lake Ontario and the eastern basin of Lake Erie about 100 yr earlier (see Schelske et al. 1983).

Although it has been documented that P limits phytoplankton production in the Great Lakes (Schelske 1979), no study has linked increased P inputs directly with increased diatom production. Therefore, we studied the Glenora-B core (Warwick 1980) to determine historical relationships between accumulation of biogenic silica (BSi), total phosphorus (TP), organic carbon (OC), and total nitrogen (TN) in the sediments. These results are particularly important because the trophic history of this core also has been studied from analyses of chironomids (Warwick

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1980) and diatoms (Stoermer et al. 1985a). Warwick (1978) also documented the demographic history and cultural events in the watershed over the 2800-yr chronology of this core to interpret the impact of human populations on the Bay of Quinte.

Materials and Methods

The Bay of Quinte on the northeastern shore of Lake Ontario is Z-shaped with a complex morphometry and surface area of 254 km^2 (Warwick 1980). All samples analyzed were from the Glenora-B core obtained 17 March 1972 from a depth of 23 m using a modified Mackereth short core sampler (Warwick 1980). This 164-cm core was sectioned at 1-cm intervals and analyzed at 1-cm intervals to 70 cm and then at 5-cm intervals to the bottom.

Warwick (1980) calculated sediment accumulation rates using a combination of techniques including the *Ambrosia* pollen horizon, radiometric dating, and an Erosion Index. The Erosion Index is based on the physical measurement of sediment grain size and is defined as the fraction of clay-sized material finer than 10.5ϕ divided by the total clay-sized fraction ($<8.5\phi$). The index was developed on the premise that one of the first manifestations of human activity in the watershed was the input of fine-grained material to the sediments. The *Ambrosia* pollen rise, initially set at 1849 ± 5 yr, was used to center the age scale developed from ^{210}Pb measurements. The Erosion Index was used to verify dates established by the pollen horizon and ^{210}Pb dating and to extend the chronology beyond the limits of the ^{210}Pb method. Based on historical interpretation of the Erosion Index, the date for the *Ambrosia* pollen rise was refined to 1852 and the chronological scale adjusted accordingly. Older sediments were dated by centering the age scale determined by ^{14}C dating on the sequence established by the other methods.

Data for TP, TN, and OC were obtained from Warwick (1980). After ignition of organic matter and digestion in HCl at 104°C for 2 h, samples for TP were analyzed in triplicate using a modified particulate P method (Stainton et al. 1974). An independent check of the TP sequence between 45.5 and 69.5 cm was conducted by J. D. H. Williams, Canada Centre for Inland Waters, Burlington, Ont., using the method of Harwood et al. (1969). Total carbon (TC) and TN were determined with a CHN analyzer. Inorganic carbon (IC) was measured using a gas stripping procedure (Stainton 1973) and subtracted from TC to obtain OC.

Samples (15–30 mg) for BSi were dried (after being stored in ethanol in glass vials), weighed, and then leached in polypropylene containers with 40 mL of 1.0% Na_2CO_3 at 85°C for 5 h in a constant-temperature bath (DeMaster 1981). After 2, 3.5, and 5 h of leaching, 1.0-mL samples were withdrawn for analysis of soluble SiO_2 on a Technicon AutoAnalyzer using the heteropoly blue method. A regression of these time-course data was extrapolated to zero time to correct for mineral interference (Krausse et al. 1983). Data were then converted to milligrams BSi per gram dry sediment.

The possibility of error introduced by Si leached from the glass vials into the ethanol solution was investigated on three samples. Although concentrations as large as 44 $mg \cdot L^{-1}$ were obtained for the ethanol solution from the 31.5-cm sample, concentrations were only 9 and 12 $mg \cdot L^{-1}$ in the 1.5- and 115.5-cm samples, respectively. Only 10 mL of liquid was present in each sample; therefore, drying the entire 31.5-cm sample would have added 0.44 mg SiO_2 to the dry weight. The total mass of sediment was approximately 500 mg so the added SiO_2 was 0.088%, only a small fraction of the 2.1 wt.% BSi determined for this sample. Because some of this added Si was probably leached from diatoms during storage, we concluded that Si leached from glass did not introduce significant errors.

Sediment accumulation rates calculated previously (Warwick 1980) were used to calculate annual accumulation rates of BSi and TP. Data on BSi and TP concentrations were multiplied by the sediment accumulation rate to obtain annual accumulation rates for each sampling interval. Data on BSi obtained by us and data on TP, TN, and OC obtained by Warwick (1980) were used to compute atomic nutrient ratios for the sediment intervals.

Results

Sediment Accumulation

Warwick (1980) found that sediment accumulation rates in the Glenora-B core varied (Fig. 1) from an average of 86 ± 9 $g \cdot m^{-2} \cdot yr^{-1}$ in sediments deposited prior to 1669 (below 67.5 cm) to 9300 $g \cdot m^{-2} \cdot yr^{-1}$ for sediments deposited in 1852–53 (37.5–35.5 cm). Initial increases in sediment accumulation leading up to this more than 100-fold increase coincided with European colonization and activity beginning with settlement by the French Sulpician Order in 1669 (Warwick 1980). Rapid deforestation in the Quinte watershed, first on the Napanee and later on the Moira and Trent rivers, probably accounted for much of the large increase in sediment accumulation preceding the major peak at 37.5–35.5 cm. Depression of the Erosion Index by increasing deposition of coarser sedimentary materials during this period of particularly high sediment accumulation indicates that the material originated near the core site, possibly from construction of milling facilities at Trenton by Gilmour and Company Lumber Industries in 1852. A secondary peak in sediment accumulation occurred between 1888 and 1905 (24.5–15.5 cm) when rates exceeded 1000 $g \cdot m^{-2} \cdot yr^{-1}$.

The magnitude of increased sediment fluxes between 1835 and 1861 (Table 1) can be illustrated by comparing linear sedimentation rates for the peak period in 1852–53 with the period before 1669. The average sedimentation rate prior to 1700 was 0.377 $mm \cdot yr^{-1}$, two orders of magnitude less than the maximum rate of 41.3 $mm \cdot yr^{-1}$ for the 6 cm of sediment (38.5–33.5 cm) that was deposited in the early 1850s (Warwick 1980).

P and BSi Accumulation

TP concentrations ranged from an average of 1.18 $mg \cdot g^{-1}$ below 67.5 cm to 2.99 $mg \cdot g^{-1}$ in near-surface sediments (Fig. 2). Five peaks in TP concentrations were found between 63.5 and 27.5 cm. The earliest peak (1.34 $mg \cdot g^{-1}$) occurred at 63.5 cm. Two major peaks of 2.10 and 2.20 $mg \cdot g^{-1}$ followed at 57.5 and 51.5 cm, respectively. In each case the major peaks were bimodal and were preceded by smaller secondary peaks of 1.49 and 1.55 $mg \cdot g^{-1}$ at 59.5 and 54.5 cm, respectively. The truncated peak between 40.5 and 29.5 cm reached a maximum of 1.77 $mg \cdot g^{-1}$ at 31.5 cm, roughly equivalent to the concentration in the fifth peak at 27.5 cm.

The complex TP profile between 69.5 and 45.5 cm was confirmed by independent analysis. Results obtained with the more rigorous methods of Harwood et al. (1969) were higher than those obtained using the method of Stainton et al. (1974), but the relative profiles were similar (Fig. 3).

The range in concentrations of BSi was greater than that for TP, increasing from an average of 8.43 $mg \cdot g^{-1}$ below 67.5 cm

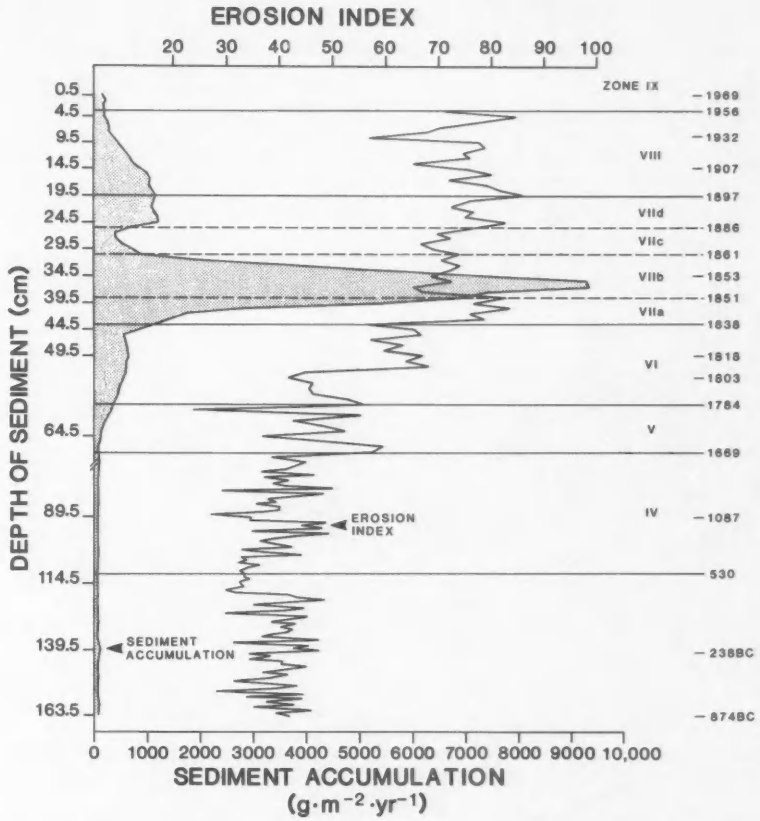


FIG. 1. Rates of sediment accumulation and the Erosion Index in the Glenora-B core, Bay of Quinte. The Erosion Index is on the right. Data taken from Warwick (1980).

TABLE 1. Comparison of sediment accumulation rates, nutrient fluxes, and nutrient ratios in sediments from the Glenora-B core, Bay of Quinte. Data are averages for the intervals specified when more than one interval is specified. Dates and zones are those used by Warwick (1980).

Depth (cm)	Date	Zone	Sedimentation rate ($g \cdot m^{-2} \cdot yr^{-1}$)	Flux ($g \cdot m^{-2} \cdot yr^{-1}$)				Nutrient ratio (atomic)
				BSi	TP	OC	TN	OC:TN:BSi:TP
1.5–2.5	1960–65	IX	21.5	23.7	0.594	18.3	2.51	83.3:9.71:21.3:1.0
3.5–7.5	1937–56	VIIIc	25.4	19.8	0.440	22.7	3.01	137:15.7:24.7:1.0
26.5–27.5	1873–1879	VIIc	413	5.05	0.675	20.7	2.19	82.2:7.47:3.93:1.0
31.5	1958	VIIb	1910	57.4	3.38	84.8	8.90	67.1:6.04:9.06:1.0
35.5	1852	VIIb	9280	129	15.2	427	47.1	74.9:7.08:4.52:1.0
35.5–37.5	1852–53	VIIb	8690	97.2	13.8	414	45.5	76.9:7.16:4.14:1.0
41.5	1843	VIIa	1750	5.80	2.38	41.5	4.07	46.6:3.91:1.29:1.0
51.5	1811	VIb	615	3.10	1.35	68.7	6.56	136:11.1:1.24:1.0
52.5–54.5	1799–1807	VIb	563	4.40	0.986	62.7	6.14	183:15.4:2.59:1.0
57.5	1788	VIa	417	2.20	0.877	47.7	4.91	145:12.8:1.32:1.0
58.5–67.5	1669–1784	V	197	1.40	0.254	21.8	2.14	244:20.5:3.16:1.0
69.5–99.5	822–1617	IV	80	0.76	0.094	10.1	1.00	286:24.2:4.17:1.0
104.5–154.5	639 B.C. – A.D. 689	I–III	88	0.69	0.104	9.42	0.93	242:20.5:3.66:1.0

to 119 $mg \cdot g^{-1}$ in the near-surface sediments (Fig. 2). However, peaks in BSi concentrations were not as apparent as those for TP. Although there is a suggestion of an increase in BSi at 63.5 cm to complement the increase in TP, the only definable peak in BSi concentration that directly corresponds to a TP peak was found at 31.5 cm.

Annual rates of BSi and TP accumulation which normalize concentration data over periods of different sediment accumula-

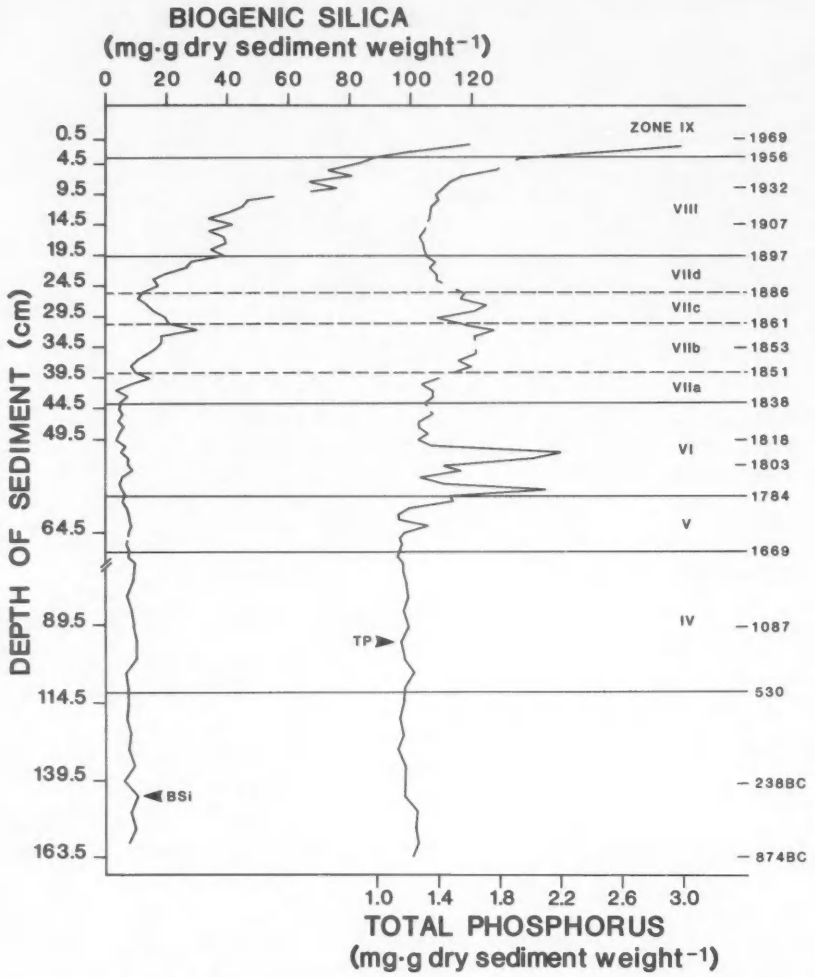


FIG. 2. Comparison of BSi and TP concentrations in the Glenora-B core, Bay of Quinte.

tion rates (Fig. 4) show that three of the main features in patterns of BSi accumulation were similar to patterns of sediment accumulation. Below 67.5 cm, BSi rates averaged only $0.71 \pm 0.09\,g \cdot m^{-2} \cdot yr^{-1}$ ($N = 22$). With the influx of sediments and 110-fold increase in sediment accumulation rates, BSi accumulation rates increased 170-fold to more than $120\,g \cdot m^{-2} \cdot yr^{-1}$ (Table 1). A secondary peak in BSi accumulation between 19.5 and 16.5 cm, which coincides with the secondary peak in sediment accumulation, averaged $43\,g \cdot m^{-2} \cdot yr^{-1}$. This rate was less than half that of the maximum and represents a smaller decrease than the eightfold decrease in mass sedimentation. Like BSi, accumulation rates of TP peaked between 36.5 and 33.5 cm with a broad secondary peak occurring between 24.5 and 15.5 cm (Fig. 4). TP accumulation rates in the peak period at 35.5 cm were approximately 150-fold greater than those in the sediments below 67.5 cm.

The quantity of BSi stored in sediments is determined by the input of BSi to the sediments, a function of diatom production and sediment focusing, and the fraction of the BSi input that is dissolved during burial (Schelske 1985). No data are available for historical rates of diatom production in the Bay of Quinte,

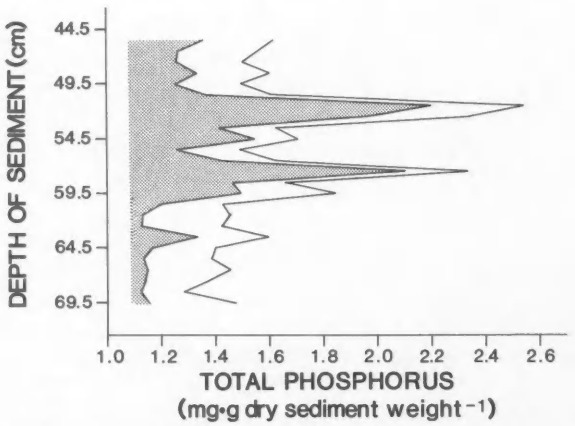


FIG. 3. Comparison of TP measurements. The curve highlighted by stipled area represents data for method of Stainton et al. (1974) and the curve on the right represents data for Harwood et al. (1969).

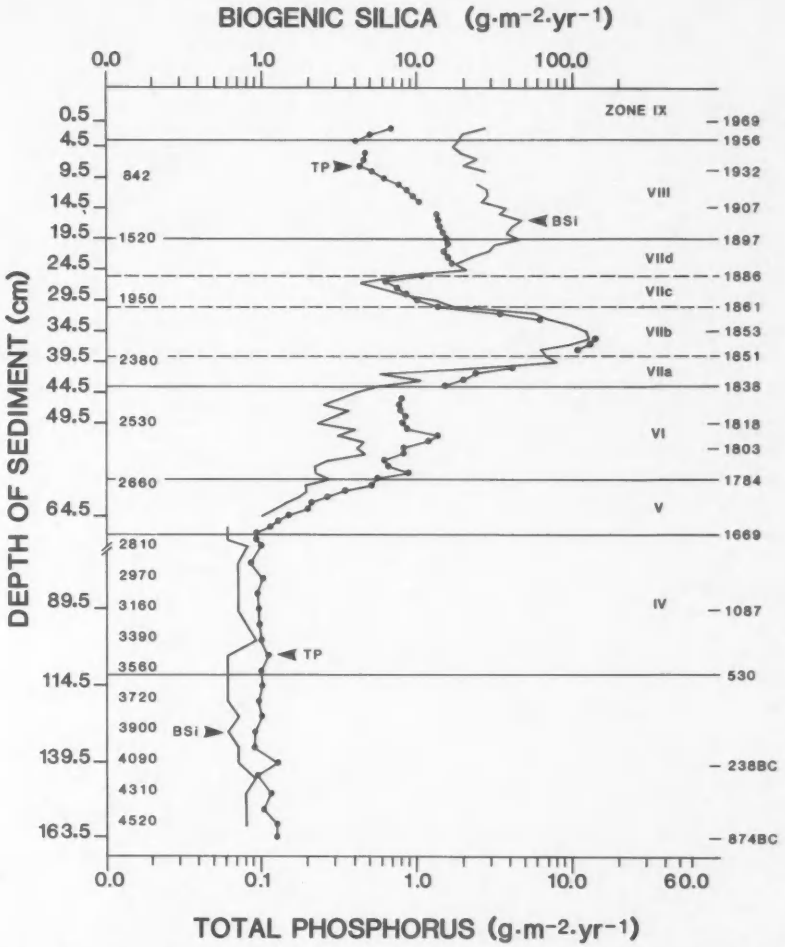


FIG. 4. Comparison of BSi and TP fluxes (log scale) in the Glenora-B core, Bay of Quinte. Numbers listed next to the depth scale are cumulative areal fluxes of BSi ($g \cdot m^{-2}$).

but in Lake Michigan, production of BSi by diatoms in the 1970s during a period of Si limitation was estimated at 89–110 $g \cdot m^{-2} \cdot yr^{-1}$ (Schelske 1985; Parker et al. 1977) of which only 5.0% or less was being stored permanently in the sediments (Parker et al. 1977). The maximum rate of BSi accumulation found in a core from Grand Traverse Bay, Lake Michigan, was 65 $g \cdot m^{-2} \cdot yr^{-1}$ (Schelske et al. 1983), an order of magnitude greater than would be expected if only 5.0% of the BSi production was permanently sedimented. The maximum accumulation rate in the Glenora-B core was even larger, being >120 $g \cdot m^{-2} \cdot yr^{-1}$. Integrated accumulation of BSi in the Glenora-B core in sediments above 39.5 cm (the *Ambrosia* horizon) was 2380 $g \cdot m^{-2}$ or an average of 19.8 $g \cdot m^{-2} \cdot yr^{-1}$ for this 120-yr period. About 350 $g \cdot m^{-2}$ of the accumulation was present in the sediment intervals between 38.5 and 30.5 cm, which, according to Warwick's (1980) chronology, accumulated at a rate of 35 $g \cdot m^{-2} \cdot yr^{-1}$ in the 10 yr between 1851 and 1861. These results indicate that the sediments at the Glenora-B site are a large sink for BSi even though the geochemical significance of these high rates in the Bay of Quinte cannot be extrapolated from the single site that was sampled.

We attribute the high rates of BSi accumulation in the Glenora-B core to the combined effects of several factors. Warwick (1978) stated that current patterns and the bed and bank configurations of the Glenora site are ideal for sediment accumulation. Diatoms produced in the upper parts of the bay as well as those produced in the lower part and adjacent waters of Lake Ontario could have been transported and sedimented, increasing the rate of BSi accumulation at the Glenora site. Stoermer et al. (1985a) found that a large proportion of the diatoms in this core were epipelic during the period before major forest clearance. In addition to the effects of transport and focusing of sediments and diatoms, diatom preservation may have been enhanced by the relatively high rates of sediment accumulation because the rapid burial of diatoms reduces the time for dissolution in near-surface sediments. As a consequence, the fraction of diatoms preserved might be relatively large in the Bay of Quinte, which, because of large P loads (Johnson and Owen 1971), would have a larger rate of diatom production than the open waters of Lake Ontario or Grand Traverse Bay. Thus, the combined effects of sediment focusing, including the transport of riverine, epipelic, and benthic di-

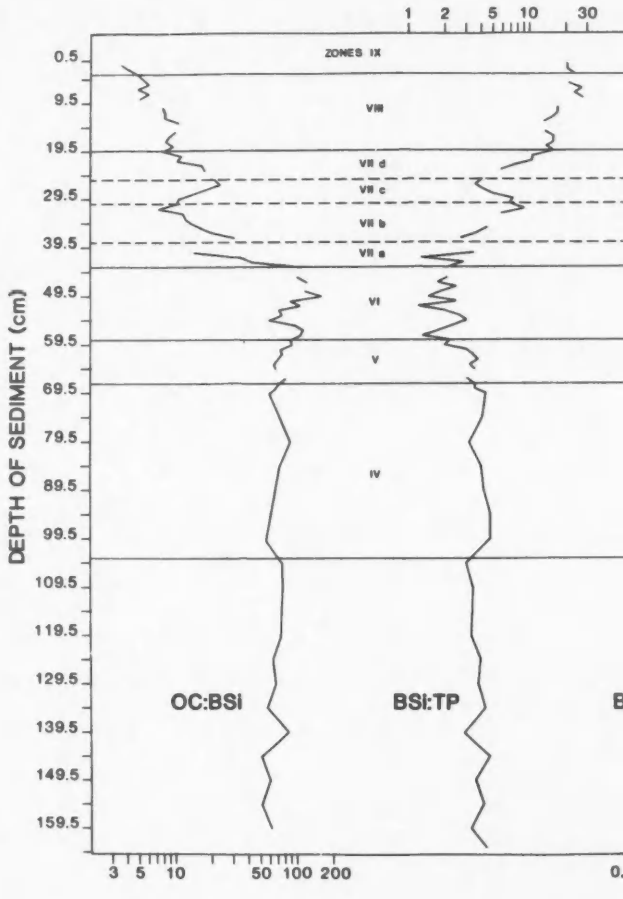
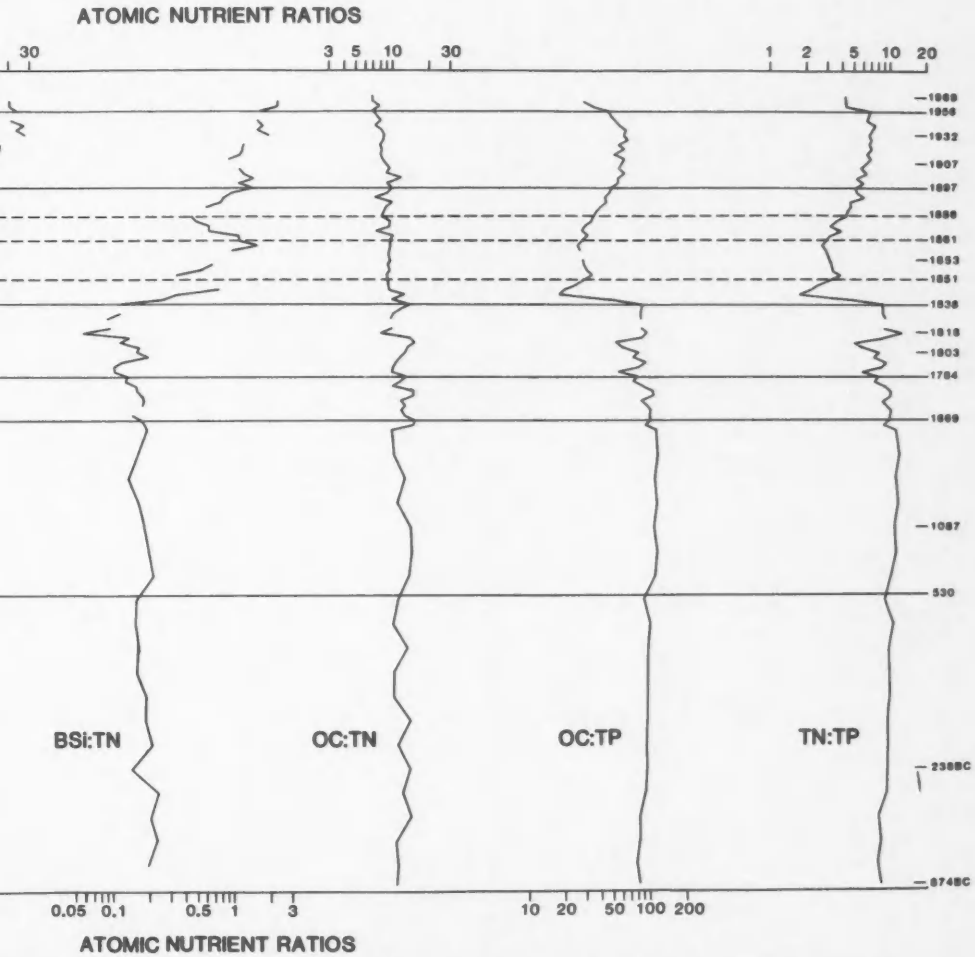


FIG. 5. Atomic nutrient ratios (log scale) in the Glenora-B c



hora-B core, Bay of Quinte. Zones listed on the right side of the graph are those described by Warwick (1980).

atoms, and high rates of production and preservation of diatoms could be significant factors in the large rates of BSi accumulation measured for the Glenora-B core.

Nutrient Ratios

Atomic nutrient ratios calculated for OC, TN, TP, and BSi demonstrate two general patterns in the sediments (Fig. 5). Each ratio was relatively constant below 66.5 cm and, with the exception of OC:TN, all changed markedly in the sediment intervals between 43.5 and 38.5 cm. The OC:TN ratio changed least of any ratio, with the largest change being a general decrease above 17.5 cm. Ratios of OC:TP and TN:TP declined to minima at 40.5 and 41.5 cm and then increased in more recent sediments except in the near-surface sediments. Ratios of BSi:TP and BSi:TN generally increased to the surface from 41.5 cm, whereas ratios of OC:BSi decreased.

The greatest changes in nutrient ratios were found in zone VII, which lasted from 1838 to 1897 (Fig. 5). During the early part of this period there was rapid population growth in the watershed. Ratios of TN:TP, OC:TP,and OC:BSi decreased greatly compared with earlier sediments whereas ratios of BSi:TN and BSi:TP increased greatly. Zone VIII, which lasted from 1897 to 1956, was characterized by the largest BSi:TP ratios in the core. The BSi:TN ratio was also high during this period, which shows that, like TP, less TN was stored relative to BSi than in earlier periods (Table 1).

Atomic nutrient ratios were normalized to TP for selected core intervals so that ranges in values could be compared over the length of the core. Ratios of BSi:TP varied about 20-fold whereas ratios of OC:TP only varied about sixfold and ratios of TN:TP only fourfold (Table 1). Ratios of OC:TN:BSi:TP in the intervals from 7.5 to 3.5 cm are roughly comparable with the Redfield ratio of 106:16:16:1 for these nutrients in oceanic particulate material (Redfield et al. 1963). Below 7.5 cm, the BSi:TP ratio was always less than the Redfield ratio. However, the pattern for OC:TP and TN:TP was different in that these ratios below 7.5 cm were less than the Redfield ratio to sediment depths >43.5 cm where the ratios nearly equalled or exceeded the Redfield ratio.

Discussion

Historical patterns of sediment storage of P, BSi, and other nutrients may be modified by diagenesis or bioturbation. Mobilization and sorption of P in sediments may confuse the expected relationship between historical patterns of P loading and sedimentation (Carignan and Flett 1981). Postdepositional mobility, however, may not affect the quantity of TP stored in the sediments. Warwick (1980) attributed the two sharp TP peaks at 57.5 cm and 52.5–51.5 cm (Fig. 2) to interference of P mobilization by precipitation with calcium carbonate and adsorption on clay minerals. Absolute quantities of BSi would be expected to decrease from dissolution of diatoms during diagenesis in the mixed sediments prior to permanent burial (see Schelske et al. 1983). Our results obtained by BSi analysis, including the very large pulses from 1852 to 1853, correspond to diatom counts made by Stoermer et al. (1985a). This correspondence indicates that the BSi analysis provides a good measure of biogenic Si in diatoms and that amorphous Si interference accounts for a relatively small fraction of the BSi reported here.

Historical events can be interpreted from the Glenora-B core because ancillary information is available on diatom (Stoermer et al. 1985a) and chironomid community structures (Warwick 1980). Data on siliceous algal microfossil assemblages indicate that the bay became eutrophic in the span of a few years in the early 1850s. These results are supported by BSi analysis (Fig. 4), but data on chironomid subfossil remains suggest a period of oligotrophication in the 1850s, which Warwick (1980) attributed to dilution and burial of food resources and development of unstable bottom conditions during periods of peak sediment accumulation. Increased turbidity resulting from soil erosion also can be presumed to have affected the community structure of primary producers. Prior to extensive forest clearance, the oligotrophic bay waters apparently were clear because the diatom stratigraphy was dominated by benthic and epipelic diatoms and by sublittoral and upper profundal zone chironomids. With changes in land use, sediment P loadings and algal production increased and the production base shifted to planktonic diatoms as epipelic and benthic diatoms and macrophytes were shaded out. Brush and Davis (1984) suggested a similar sequence of historical changes in primary producer communities in oligohaline and mesohaline sections of Chesapeake Bay.

Our results show that P inputs directly affected Si dynamics in the Bay of Quinte. The earliest increases in BSi and TP accumulation rates were found during the French Period (zone V), which began in 1669 (Fig. 4). BSi and TP accumulation rates increased steadily from 1669 to 1784, less regularly through the early stages of colonization from 1784 to 1838 (zone VI), and then much more rapidly as settlement and activity in lumbering, particularly for the American market, accelerated. Lumbering activity reached its greatest intensity in the 1850s and 1860s (zones VIIb and VIIc) when the watershed was stripped of most of its timber resources. After 1853, BSi and TP accumulation decreased sharply, and then from 1886 to 1897 (zone VIId) BSi accumulation increased rapidly while TP accumulation decreased slightly. This pronounced shift in relative rates of BSi and TP accumulation occurred during a period when lumbering activity decreased and population declined (Warwick 1980). Redistribution of population to urban centers and changes in agricultural practices probably account for the relative increase in BSi accumulation rates even though population numbers generally were declining. Increases in P inputs could be expected as farming practices changed from subsistence agriculture to an enormous export demand for agricultural products (particularly wheat and dairy produce) during the 1860s (P. G. Sly, Canada Centre for Inland Waters, Burlington, Ont., unpubl. data).

Although rates of BSi and TP accumulation were closely coupled and related to sediment accumulation rates, chronological patterns of TP and BSi differed. Prior to 1669, neither rate varied much, with BSi accumulation being about fivefold greater than TP (Fig. 4). During the period of early European settlement, TP accumulation increased more than BSi accumulation, a pattern that persisted from 1669 to 1838 (zones V and VI) when BSi accumulation was two- or three-fold greater than TP (Table 1). This pattern changed in zone VII during the period of maximum accumulation in 1852–53 when BSi accumulation rates increased and were about 10 times greater than those for TP. Rates of BSi accumulation after 1888 were 30–40 times greater than TP, partly as a result of the greater relative decrease in TP accumulation.

An increase in accumulation rates of BSi after 1888 is also evident from changes in the ratio of BSi:TP (Fig. 5). In sediments deposited after 1888 (above 19.5 cm) the BSi:TP

ratio ranged from 20:1 to 40:1, whereas in the sediments below 69.5 cm the ratio averaged <4.0:1. The relative increase in BSi accumulation after 1888 when sedimentation rates decreased provides strong evidence that increases in BSi accumulation rates were one consequence of nutrient enrichment in the Bay of Quinte.

This relative increase in BSi fluxes after 1888 may have been one consequence of constructing sewage collection systems at major cities along the shoreline (Trenton and Napanee about 1890 and at Belleville by 1900; P. G. Sly, pers. comm.). Sewage effluents contain a relatively large fraction of available P. Therefore, effluents discharged directly to the bay from an increasing urban population could have increased not only the TP loading, but also the proportion of TP that was available for diatom growth. Studies of sediment cores from Lake Erie (Williams et al. 1976) and several lakes in France (Vernet and Favarger 1982) have shown that the proportion of available P increased in recent sediments whereas the concentration of apatite P, a nonavailable form, was relatively constant with sediment depth. Construction of sewage collection systems followed later by the introduction of phosphate detergents probably increased the fraction of combined TP inputs that was available for phytoplankton growth and increased the production of diatoms and accumulation rates of BSi relative to TP accumulation.

The dynamic nature of the Bay of Quinte, including the relatively short hydraulic residence time and exchange of water with Lake Ontario, and the lack of historical information on Si dynamics make it difficult to confirm the onset of Si depletion with data from the Glenora-B core. P inputs prior to the period of maximum BSi accumulation in the early 1850s probably were not great enough to stimulate diatom production to the extent that it was Si limited (Fig. 4). Si-limited diatom growth probably would have been transient or seasonal because Si supplies could have been renewed rapidly from tributary inputs in this bay in which flushing occurs rapidly, especially during the winter and spring (Johnson and Owen 1971). In addition, Si supplies could have been renewed periodically by the summer subsurface flow of cold water from Lake Ontario (P. G. Sly, pers. comm.). The large increase in TP loading beginning in the 1840s undoubtedly stimulated diatom growth and caused the peak in BSi accumulation in 1852–53. Increased diatom production during this period may have rapidly utilized available Si supplies, causing seasonal Si depletion and Si-limited diatom growth in the water column and decreased BSi inputs to the sediments. This sequence of events has been postulated to explain the distribution of BSi (Schelske et al. 1983) and diatoms (Stoermer et al. 1985b) in a core from Lake Ontario and could have occurred also in the adjacent Bay of Quinte.

The following prolonged period of high BSi fluxes from 1888 to 1907 could have resulted from partial renewal of Si supplies in the water column (a consequence of the preceding period of decreased TP inputs), by increased TP inputs or increased P availability that enabled diatoms to utilize a greater fraction of Si in the water column, from an increase in the fraction of diatoms that were preserved in the sediments, or by a combination of these processes. Relatively large concentrations of available P during spring mixing apparently account for the present severe Si depletion (annual range in concentration from 0.1 to 0.5 mg $SiO_2 \cdot L^{-1}$) in Lake Ontario and the central and eastern basins of Lake Erie (Schelske et al. 1985) and the predominance of stenothermal, benthic, and nearshore planktonic diatom populations in the sediments deposited after 1945 in Lake Ontario (Stoermer et al. 1985b). Obviously this type of severe Si depletion can occur only if available P supplies are large enough to support levels of diatom production during vernal mixing that exhaust or deplete Si supplies before the water column is thermally stratified. We believe that diatom production was maximized after 1888 in the Bay of Quinte in response to an increased proportion of available P in combined TP loadings and to increased water clarity.

Other paleolimnological results from the Great Lakes strongly support the results obtained from the Glenora-B core. Harris and Vollenweider (1982) attributed changes in diatom species composition in a core from the central basin of Lake Erie to nutrient enrichment in the mid-1800s. Studies of BSi storage in cores from Lake Erie and Lake Ontario also show the early effects of eutrophication during the 1800s (Schelske et al. 1983). Our results and those of Stoermer et al. (1985a) for the Bay of Quinte are more dramatic in this respect because they show increased storage of BSi and changes in diatom species composition in the sediments, possibly as early as the late 1600s during the French Period when human impacts in the watershed were minimal. These results point to the extreme sensitivity of phytoplankton assemblages in the Laurentian Great Lakes to P and other nutrient enrichment accruing from anthropogenic activities in the watershed and to the value of using the sediment record to study these changes.

Acknowledgments

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Changes in Territorial, Gill-flaring, and Feeding Behavior in Juvenile Coho Salmon (*Oncorhynchus kisutch*) following Short-term Pulses of Suspended Sediment

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The territorial, gill-flaring, and feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*) in a laboratory stream was disrupted by short-term exposure to suspended sediment pulses. At the higher turbidities tested (30 and 60 nephelometric turbidity units (NTU)), dominance hierarchies broke down, territories were not defended, and gill flaring occurred more frequently. Only after return to lower turbidities (0–20 NTU) was social organization reestablished. The reaction distance of the fish to adult brine shrimp decreased significantly in turbid water (30 and 60 NTU) as did capture success per strike and the percentage of prey ingested. Implications of these behavioral modifications suggest that the fitness of salmonid populations exposed to short-term pulses of suspended sediment may be impaired.

Le comportement territorial et alimentaire ainsi que le déploiement des opercules chez des saumons cohos (*Oncorhynchus kisutch*) juvéniles stabulés dans un cours d'eau expérimental ont été perturbés par l'exposition à court terme à des séries d'apport de sédiments en suspension. Aux plus fortes turbidités étudiées (30 et 60 unités nephelométriques de turbidité (NTU)), les hiérarchies de dominance se sont effondrées, les territoires n'ont pas été défendus et le déploiement des opercules s'est effectué plus souvent. Ce n'est qu'après le retour à de plus faibles turbidités (de 0 à 20 NTU) que l'organisation sociale a été rétablie. En eau turbide (30 et 60 NTU), la distance de réaction du poisson aux artémias adultes a nettement diminué tout comme le succès de capture par attaque et le pourcentage de proies consommées. Ces modifications comportementales portent à croire que la santé des populations de poisson exposées à court terme à des séries d'apport de sédiments en suspension peut être altérée.

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The acute and chronic effects of long-term exposure to suspended sediment on fish are well documented (Crouse et al. 1981; Gardner 1981; Sigler 1981; Noggle 1978; Confer et al. 1978; Vinyard and O'Brien 1976; Rogers 1969; Herbert and Merkens 1961; Wallen 1951). Surprisingly little is known about behavioral effects of short-term intermittent exposure. For those species of fish inhabiting streams in watersheds subjected to logging (trouts, some juvenile salmon), brief "pulses" may represent the type of exposure to sediment most frequently encountered (Reid 1981).

Juvenile coho salmon (*Oncorhynchus kisutch*) are territorial drift feeders (Mundie 1969; Hartman 1965; Mason and Chapman 1965; Chapman 1962). Territoriality has long been recognized as a mechanism for limiting the density of stream-dwelling salmonids and as a characteristic evolved as a food-linked spatial requirement (Slaney and Northcote 1974; Jenkins 1969; Chapman and Bjornn 1969; Allen 1969; Kalleberg 1958). A consequence of repeated disruption of territoriality may be decreased fish production.

Our purpose was to determine the direct effect of exposure to a short-term pulse of suspended sediment on the behavior of juvenile coho salmon in a laboratory stream, simulating natural field conditions.

Methods

Juvenile coho salmon were tested because young of this species spend at least 1 yr in small streams before migrating to the ocean. Consequently, young coho are exposed much longer to effects of suspended sediment than are young pink salmon (*Oncorhynchus gorbuscha*), which migrate downstream immediately after emergence. The test fish were seined from the Salmon River, British Columbia (49°07′N, 122°35′W), transported to laboratory facilities at the University of British Columbia, and held in 200-L fiberglass tanks. Holding conditions included a flow-through water filtering system, a 12-h photoperiod, and a water temperature of 7.0 ± 0.5°C. Fish were fed a mixture of frozen adult brine shrimp, tetra-min R, and ground liver twice daily (08:30 and 16:30).

The experimental apparatus consisted of an oval Plexiglas trough which simulated a stream channel. Fish were restricted to one of the straight sections of the trough by 1-cm-mesh screens. This produced an observation area 100 × 25 × 25 cm. Water depth was 25 cm. A refrigeration unit maintained the water temperature at 10.0 ± 0.5°C. Light was supplied by two 2.5-m fluorescent lights (Duro-Test Vita Lites) suspended above the trough. Light intensity, measured with a Li-cor photometer at a

depth of 0.5 cm below the surface, was 2.6, 0.9, 0.3, and 0.1 $\mu E \cdot m^{-2} \cdot s^{-1}$ at turbidities of 0, 20, 30, and 60 nephelometric turbidity units (NTU), respectively. Water velocity was measured with a Nixon Instruments Streamflow Flowmeter (series 400). Maximum water velocities (18 $cm \cdot s^{-1}$) were measured at the upstream end of the observation area. Downstream velocities averaged 10 $cm \cdot s^{-1}$. Oxygen concentration was maintained near saturation by airstones. The bottom of the observation area was covered with gravel (~2.5 cm in diameter) and several large stones (~8.5 cm diameter). These were collected from a stream and washed clean.

The experimental channel was enclosed in a curtain of black plastic to prevent disturbance to the fish. Observations were made through a small slit in the curtain.

The sediment used to create the suspended sediment pulses was collected from a settling pond at a gravel pit alongside the Coquitlam River (48°19′N, 122°45′W). Logging companies acquire road fill materials from such sources; hence, this sediment was a reasonable representation of the fine-grained particles contributed to streams by roads in logged watersheds (Reid 1981). The sediment was wet-sieved through a 0.063-mm screen and the portion passing through was used to produce the suspended sediment pulse. This fraction was analyzed by the hydrometer technique of Day (1965) and was largely composed of particles ranging between 0.02 and 0.06 mm, with a low percentage of particles smaller than 0.01 mm (Fig. 1). Scanning electron micrographs revealed very angular sediment particles, averaging 1.5 on a particle roundness scale of 0–6, angular to round, respectively (Blatt et al. 1972).

The suspended sediment pulse was produced by mixing the sediment with water to form a thick aqueous slurry which was allowed to settle for 30 min. The suspended fraction was then slowly decanted into a header box situated over the experimental stream trough. Enough slurry was allowed to flow into the channel until the desired turbidity was attained. Turbidity was measured at three depths (0, 12, and 25 cm) at each of four stations by the standard optical technique (APHA 1975). A Fisher 400 DRT turbidimeter was used to measure turbidity in nephelometric turbidity units (NTU).

The response of the fish to a short-term pulse of suspended sediment was tested by recording their territorial and feeding behavior in the observation area of the stream channel during nonturbid conditions (pretreatment phase), during turbid conditions (treatment phase), and for a period following its clearance (posttreatment phase). As the rate of sediment pulse may affect the responses of fish to suspended sediment, two groups of eight fish were each subjected to a sharp increase in turbidity (over 1 h) to the maximum tested (60 NTU), and a third group was exposed to a gradual increase (over 2 d) to the same maximum turbidity level. The sediment dynamics during the descending limb of the sudden and gradual sediment pulses spanned a similar period of time (2.5 d). The length of the pretreatment phase (2.5 d) and the posttreatment phase (2 d) was identical in the sudden and gradual sediment pulse experiments.

Individual groups of eight fish (territorial tests) and five fish (feeding tests) were randomly selected from the holding tanks for each experiment and its replicate. The test fish were anaesthetized with 2-phenoxyethanol and their length and weights recorded (mean length = 5.2 mm, range = 4.7–6.0 mm; mean weight = 1.7 g, range = 1.1–2.8 g). A small portion of the caudal fin of each fish was clipped off to allow individual identification. The test fish were then placed into the observation area and allowed to acclimate for at least 5 d. The development of a stable pattern of social behavior and commencement of feeding was used as the criterion for acclimation.

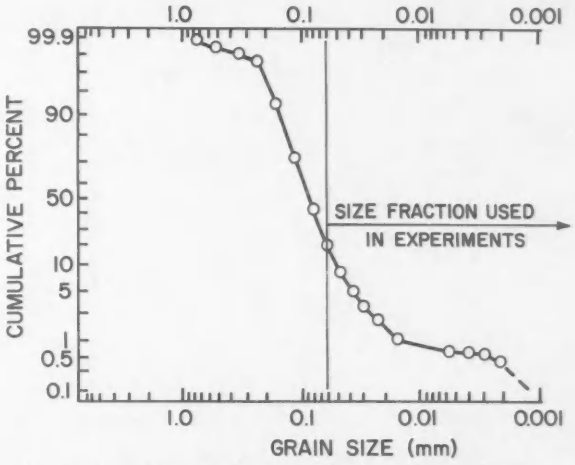

FIG. 1. Logarithmic grain size distribution of the test sediment.

In the experiments investigating the effect of short-term exposure to suspended sediment on territorial behavior, each fish was observed in random order for five 2-min trials during each of seven to nine observation periods per phase. Observations were made between 07:30–11:30 and 13:30–16:30. The behavioral data were collected on a MORE 0S-3 Behavioral Event Recorder and were supplemented by observer notes. In addition, the frequency of gill flaring was recorded during each experimental phase. Gill flaring was recognized as an excessive opening of the gill opercula, gaping mouth, and a small but distinct forward movement of the fish.

Four classes of fish were recognized within the heirarchial structure established: territorial, partially territorial, defensive, and submissive. Territorial fish defended their territories (as defined by Noble 1939) against all fish whereas partially territorial fish only defended their territories against fish subordinate to them (Collias 1944; Greenberg 1947). Defensive fish attempted to displace others from their immediate area, but were not always successful. Submissive fish were easily "intimidated" by the approach of other fish and also by interactions occurring between other fish. Submission was characterized by a compression of all fins, dark coloration, and movement away from other fish. The behavioral data were analyzed with a Mann–Whitney U-test and considered significant at $p < 0.05$.

For the feeding experiments, frozen adult brine shrimp were introduced to the trough through a funnel and tube, 10 cm upstream from the observation area. The prey invariably followed a trajectory along the centre of the channel at a depth of about 10 cm from the surface at the upstream end, 8 cm near the middle, and 1.5 cm at the downstream end of the observation area. Due to the dominance hierarchy and territorial behavior of the fish, the dominant fish, positioned upstream of the subordinates, consumed all prey items when they were introduced one at a time. Therefore, in order to alleviate interfering variables such as hunger difference among fish, shrimp were provided in excess at the end of each day. Prey items that were not ingested were collected in a net immediately downstream from the observation area.

A total of five trials, each involving 10 introductions of a single prey item, were run during each experimental phase

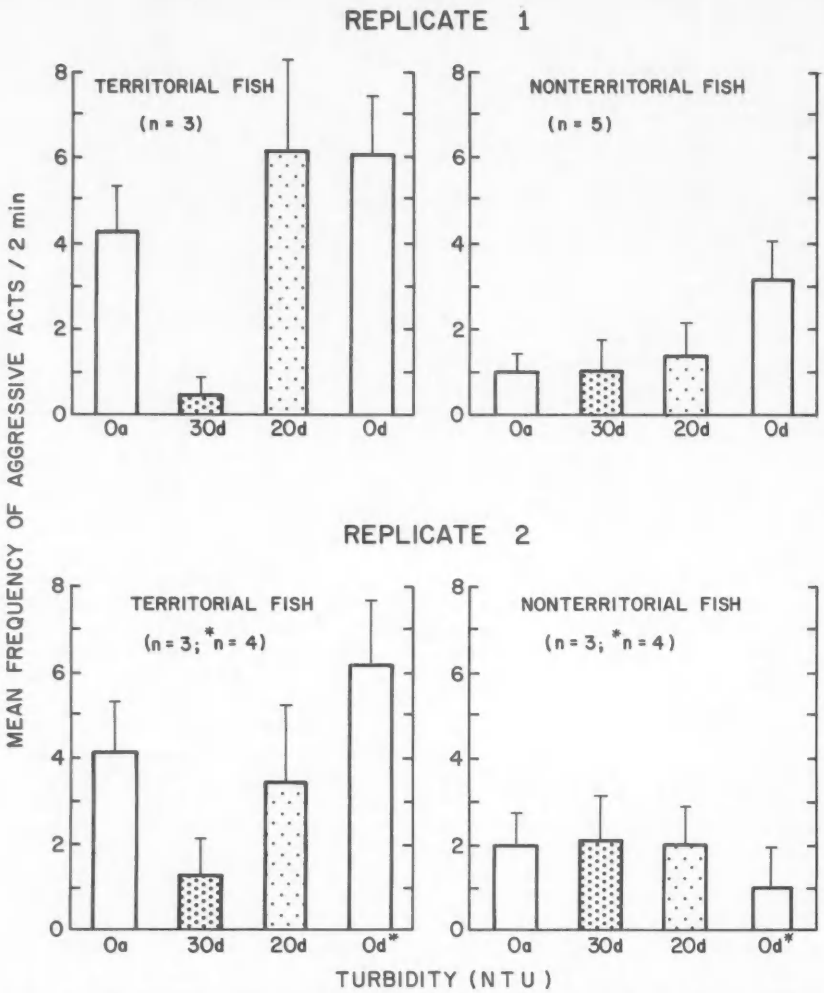

FIG. 2. Frequency of aggression by juvenile coho salmon during the sudden pulse experiments. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse; numbers of fish given in parentheses.

(pretreatment, treatment (60, 30, 20 NTU), and posttreatment). Prior to prey introduction, the holding position of each fish was recorded. Following the addition of the prey item, the identity of the captor was recorded as well as the site of prey capture, assigned to one of two catagories: 1, upstream from the holding position; 2, downstream from the holding position. These observations also permitted the reaction distance of the fish to be determined as well as the number of strikes per prey capture. The data were subjected to a t-test and considered significant at $p < 0.05$.

Results

Territoriality

Sudden pulse

A dominance hierarchy and several territories were established during the period of acclimation. One fish was dominant over all others, a second was subdominant, and three additional levels of social rank were present amongst the subordinate fish. Territory establishment was related to social rank. Both the dominance hierarchy and the territories were stable throughout the pretreatment phase. The holding positions of the fish were for the most part located between depths of 3 and 7 cm from the surface.

During the pretreatment phase, the territorial fish exhibited a significantly greater number of aggressive acts than nonterritorial fish (Fig. 2). This aggression was associated with territory defense whereas the aggression of the nonterritorial fish was exhibited in response to an aggressive act directed at them.

The sediment slurry added to the channel spread rapidly downstream with the flow in the stream channel; when it first entered the observation area, most fish swam upstream to investigate its leading edge. Then they drifted downstream, remaining in the clear water until confined within turbid water by the downstream screen. The few fish that were visible

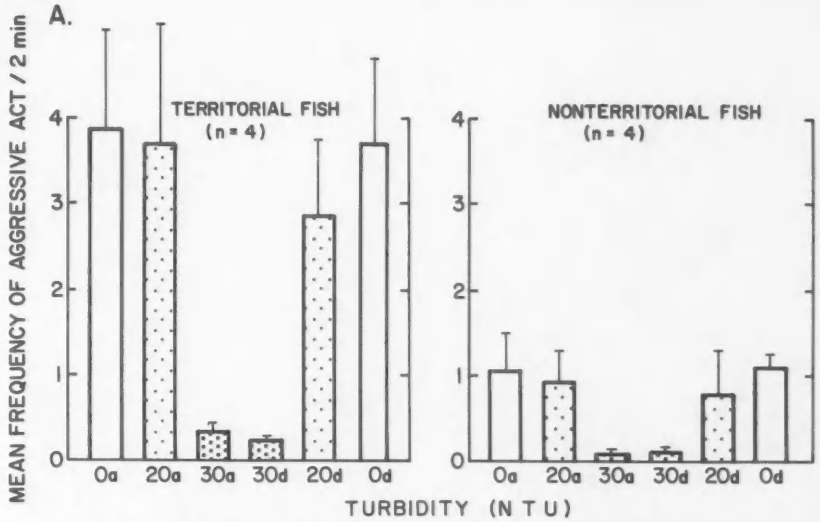

FIG. 3. Frequency of aggression by juvenile coho salmon during the gradual pulse experiment. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse; numbers of fish given in parentheses.

appeared "alarmed." Some swam in sporadic spurts throughout the observation area; others entered the gravel and remained there for several hours. Detailed observation of fish behavior was difficult at the highest turbidity tested (60 NTU) but the few interactions between fish that could be observed suggested that both the dominance hierarchy and territorial behavior were disrupted. Formerly dominant individuals no longer exerted their dominance, and no fish were observed to defend a territory. The "alarm" reaction lasted approximately 3 h, and by the end of the fourth hour of observation following sediment introduction, periods of activity were infrequent. Fish in the gravel remained there.

Following a decrease in turbidity to 30 NTU, behavioral observations again could be quantified and individual fish identified. The dominance hierarchy was less structured. Only two of six fish retained their social rank. No territory defense was observed. Formerly dominant fish exhibited significantly fewer aggressive acts than during the pretreatment phase (Fig. 2), but there was no change in frequency of aggression by nonterritorial fish. After the relatively small decrease in turbidity (from 60 to 30 NTU), fish had moved out of the gravel but remained within the lower 10 cm of the water column.

With a subsequent decrease in turbidity to 20 NTU, the dominance hierarchy became more structured. In each replicate, the dominant fish reestablished a territory and other fish also attempted to secure a territory. Consequently, the frequency of aggression by the territorial fish increased during this phase, significantly so in replicate 1 (Fig. 2).

During the interval between the 20 NTU and posttreatment phases, dominant–subordinate relationships were reorganized and additional territories reestablished. This social organization, which was similar to that observed during the pretreatment phase, was stable throughout the posttreatment phase. The territorial fish exhibited aggression significantly more frequently than the nonterritorial fish (Fig. 2) and at a slightly increased rate compared with that observed during the pretreatment phase. With further reduction in turbidity to nearly 0 NTU, the fish extended their holding positions to locations higher in the water column (5–20 cm below the surface).

Gradual pulse

The behavior of the fish during the pretreatment phase of this set of experiments was similar to that observed during the same phase in the sudden pulse experiments. The dominance hierarchy and territories established during the period of acclimation were stable throughout the pretreatment phase. Similarly, the holding positions of the fish were located between depths of 5 and 20 cm. Territorial fish exhibited a significantly greater number of aggressive acts than nonterritorial fish (Fig. 3) and all aggression was related to territory defense.

The gradual introduction of suspended sediment to produce a turbidity of 20 NTU did not alter the behavior of the fish. There was no "alarm" reaction as occurred when fish were exposed to a sudden increase to 60 NTU, no shifts in their holding positions, no disturbance of the dominance hierarchy, nor any change in the aggressiveness of the two classes of fish (Fig. 3). A further increase in turbidity to 30 NTU did produce a major disruption in the social organization of the fish. The dominance hierarchy, which had consisted of six levels, was now only composed of two. The formerly territorial fish were not observed to defend territories; consequently, the frequency of aggressive behaviors exhibited by these fish was significantly lower than during the previous phases (Fig. 3). No "alarm" reaction was observed. Fish, however, did lower their holding position to within 10 cm of the gravel after the increase in turbidity. No "alarm" reaction was observed even when the maximum turbidity tested (60 NTU) was reached. The behavior of the fish was similar to that in the previous phase and did not change when the turbidity decreased from 60 to 30 NTU. Subsequent decrease in turbidity to 20 NTU resulted in reformation of several dominant–subordinant relationships. Three of the four previously territorial fish also reestablished territories. Consequently, the frequency of aggression by the territorial fish increased significantly (Fig. 3). With the decrease in turbidity, fish moved higher into the water

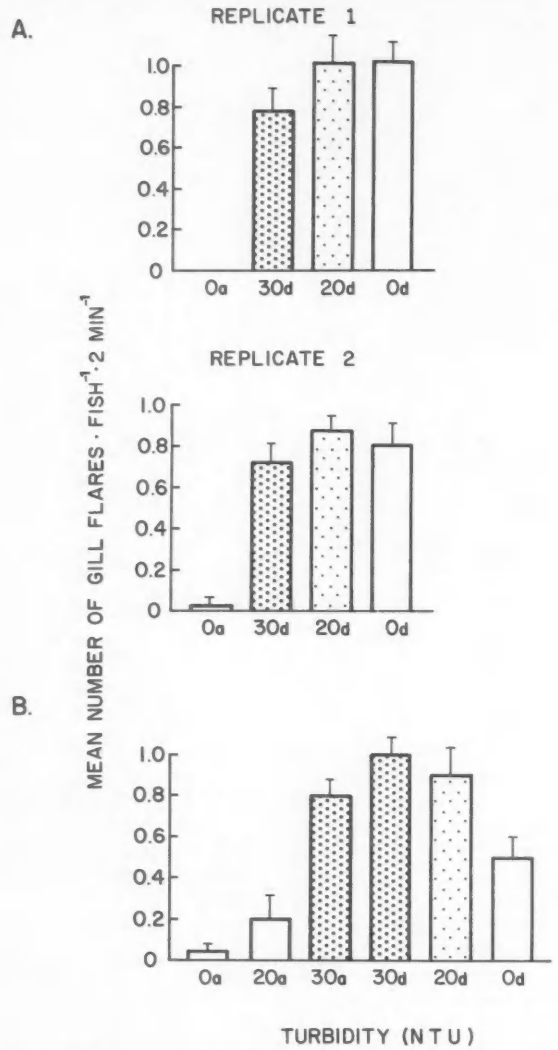

FIG. 4. Frequency of gill flaring by juvenile coho salmon during (A) sudden pulse and (B) gradual pulse experiments. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse.

column. Fish behavior during the posttreatment phase was similar to that prior to exposure to a sediment pulse.

Gill Flaring

Gill flaring was infrequent during the pretreatment phases of both the sudden and gradual pulse experiments (Fig. 4A, 4B). After the sudden sediment pulse attained a maximum turbidity of 60 NTU, the few fish visible to the observer increased their frequency of gill flaring. Significantly increased levels of gill flaring were noted during the 30 and 20 NTU phases (Fig. 4A) and remained elevated during the 3-d posttreatment phase.

The gradual addition of suspended sediment to 20 NTU produced a small but significant increase in frequency of gill flaring (Fig. 4B). Not until turbidity increased to 30 NTU did the frequency of gill flaring approximate that observed during the sudden pulse experiment. Gill flaring remained elevated with

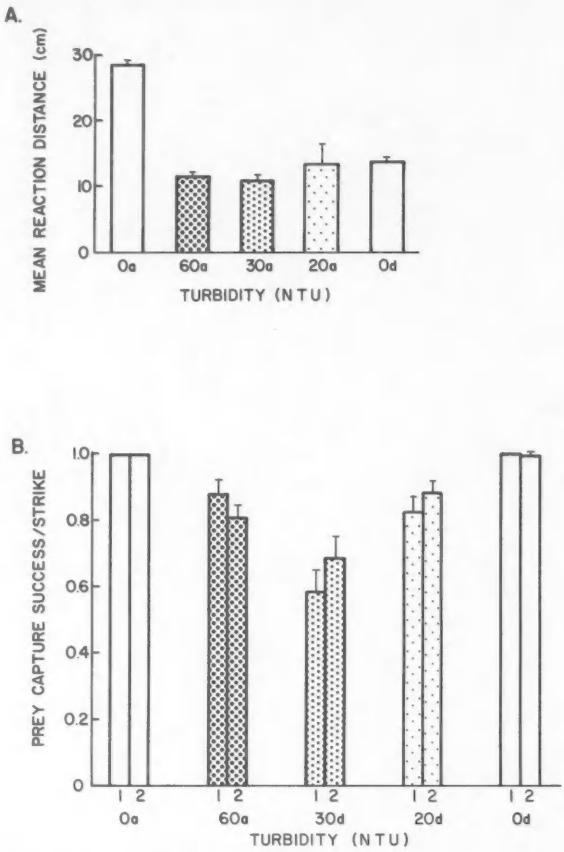

FIG. 5. Effects of turbidity on the ability of juvenile coho salmon (replicates 1 and 2) to capture prey. (A) Mean reaction distance; (B) prey capture success per strike. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse.

prolonged exposure and did not decrease significantly until the posttreatment phase.

Feeding

During the pretreatment phase when no turbidity was present, the mean reaction distance of the fish in capture of adult brine shrimp was nearly 30 cm (Fig. 5A). This distance decreased significantly to values near 12 cm during the turbid phases (60, 30, and 20 NTU) of the experiment and did not increase significantly during the posttreatment phase.

During the pretreatment phase, every strike at a prey item resulted in successful capture (Fig. 5B). Prey capture success decreased significantly during the sediment pulse (60–20 NTU) and was lowest during the 30 NTU treatment phase (Fig. 5B). The success rate per strike during the posttreatment phase was virtually identical to the pretreatment phase (Fig. 5B).

Almost all prey were captured upstream of the holding position of the captor during the pretreatment phase (Fig. 6A). Significantly lower percentages of prey (16.3, 10.8, and 16.2) were captured at upstream sites during the 60, 30, and 20 NTU treatment phases, respectively (Fig. 6A). Prey taken upstream of the captor during the posttreatment phase increased significantly but still was significantly lower than pretreatment values.

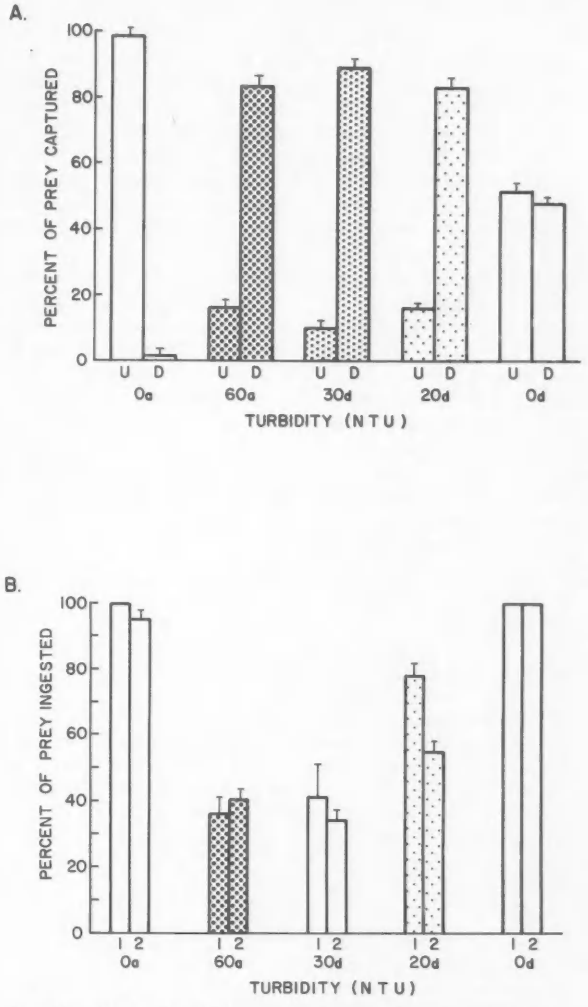

FIG. 6. Effects of turbidity on the ability of juvenile coho salmon (replicates 1 and 2) to capture prey. (A) Prey capture site: U = upstream of captor, D = downstream of captor; (B) percent prey ingested. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse.

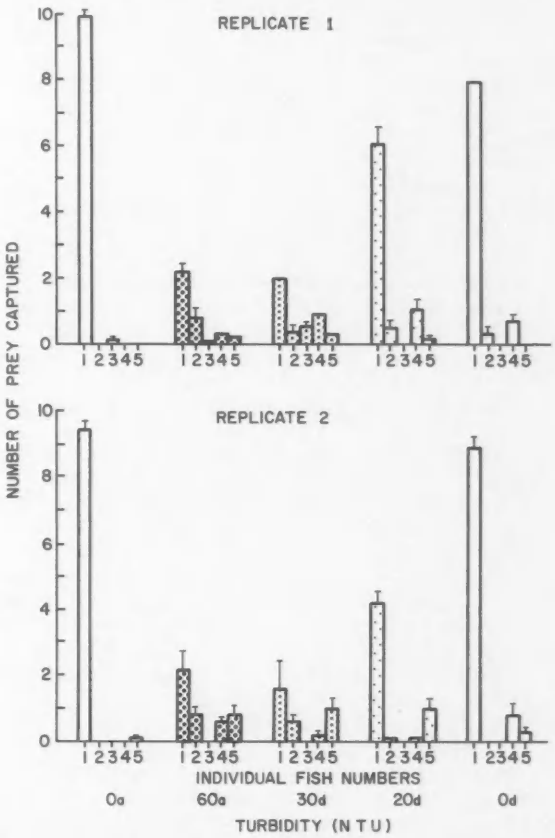

FIG. 7. Prey consumption by individual juvenile coho salmon. Vertical lines show ±1 SE; a = ascending limb of pulse; d = descending limb of pulse; 1 = dominant fish; 2–5 = subordinate fish.

Nearly all prey introduced into the channel during the pretreatment phase were ingested by the fish (Fig. 6B). Ingestion rates significantly decreased to well below 50% at the higher turbidities tested (30 and 60 NTU). Prey acquisition increased significantly during the 20 NTU phase but was well below levels observed during both the pre- and posttreatment phases.

The disruption in social organization of the fish by the suspended sediment pulse also altered their feeding behavior. During the pretreatment phase, the dominant fish, positioned upstream of the others, consumed the majority of prey items (Fig. 7). During the turbid phases, when the social organization was unstable, subordinate fish consumed a greater proportion of the total number of prey captured. This tendency was most evident at the higher turbidities (30 and 60 NTU). With the reestablishment of the dominance hierarchy, the despot again consumed most of the prey.

Discussion

Our laboratory experiments indicate that territorial and feeding behaviors of juvenile coho salmon are affected by exposure to short-term pulses of suspended sediment. In doing so, they suggest that the fitness of juvenile fish frequently subjected to such suspended sediment pulses may be impaired. Territories not only provide fish with the advantage of a greater feeding opportunity (Fenderson et al. 1968; Jenkins 1969), and consequently larger body size than nonterritorial fish (Chapman 1962; Mason and Chapman 1965), but may also reduce energy expenditures, predation, and risk of movement to less favorable habitats.

The social organization necessary for territoriality, and the feeding processes of juvenile coho salmon, are strongly influenced by visual contact (Jenkins 1969; Mundie 1969). Visual isolation may affect these behaviors (Fabricius and Gustafson 1954; Kalleberg 1958; Dill 1978). Jenkins (1969) showed that the presence of a fish within the visual range of another constitutes a threat in itself. In the experiments reported herein, turbidity provided the visual isolation neccessary to disrupt social integration. When the fish presumably were no longer within the visual range of each other (30 and 60 NTU phases), dominance hierarchy and territorial behavior were disrupted but reformed once again during 20 NTU and posttreatment phases.

A reduction in the ability of fish to visually locate prey in the higher turbidity water is supported by the results of our measurements of reaction distance and also by other researchers (Gardner 1981; Confer et al. 1978; Vinyard and O'Brien 1976). In addition, the shift in holding position of the fish from the water column to the stream bottom as turbidity increased may have been related to visual impairment. Fish use visual contact with stream bank and bottom to maintain position in flowing water. Therefore, they may have had to move closer to the bottom at the higher turbidities in order to make visual or tactile contact.

Juvenile coho salmon are visual drift feeders (Mundie 1969). Any decrease in their reaction distance, as occurred with increased turbidities, decreases the volume of water that a fish can effectively search for prey items. Therefore, the availability of prey to fish is indirectly affected by turbidity.

The effect of turbidity on the social behavior of juvenile coho salmon is also evident in their feeding behavior. Dominant territorial fish suppress the feeding activity of subordinate fish (Jenkins 1969; Symons 1968; Chapman 1962). Fenderson et al. (1968) showed that dominant Atlantic salmon (*Salmo salar*) eat twice the amount of food as subordinate fish. When the dominant was removed and replaced by a fish of a lower social status, the amount of food eaten by the subordinates increased. In the experiments reported herein, removal of the dominant was mimicked by dominance suppression resulting from turbidity. When turbidity decreased, the social hierarchy of the fish was reestablished, and the dominant fish suppressed the feeding activity of the subordinates once again.

Agonistic interactions do, however, decrease the time available for feeding. Social interactions were responsible for low feeding rates in hatchery Atlantic salmon (Fenderson and Carpenter 1971). This effect was also seen in our experiments. The low percentage of prey captured by fish in replicate 2 during the 20 NTU phase may be attributable to the occurrence of a greater number of interactions in comparison with replicate 1. Similarly, the high percentage of prey caught downstream of the captor during the posttreatment phase, as well as the reduced reaction distance of the fish during the nonturbid posttreatment phase, also could be a result of agonistic interactions. These interactions directed the attention of the fish away from the upstream end of the observation area where the prey were introduced. Consequently, fish often seemed to be unaware of prey until they were nearby. Therefore, the ability of the young coho to feed was not only decreased by the direct effect of turbidity upon their vision but also by its indirect effect on their territorial behavior.

The increased gill flaring observed during and following a sediment pulse may have resulted from gill irritation. The angularity of the sediment particles may have irritated the gill membranes and/or become lodged between the gill filaments, resulting in the production of mucus (Noggle 1978; Herbert and Merkens 1961). The mucus and any adhering sediment particles, are then flushed out of the gills as a result of their flaring action. In the gradual pulse experiment, the frequency of gill flaring did not approximate that observed in the sudden pulse experiment until similar turbidities and hence sediment concentrations were reached.

Pulse dynamics appeared to be important in affecting the initial response of the fish to suspended sediment. A gradual increase in turbidity did not produce an "alarm" reaction as was evident with a sudden increase. In the field the "alarm" reaction may bring about a displacement of fish downstream, away from the sediment source. McLeay et al. (1984) reported a downstream movement of Arctic grayling (*Thymallus arcticus*) in response to a sediment pulse. If no "alarm" reaction occurs with a gradual increase in turbidity, as may be the case in rainfall events in stream watersheds, then downstream displacement of fish may be minimal. Sudden pulses of suspended sediment, as occur from logging practices (soil failures, across stream yarding, stream machinery crossing), may displace fish into new habitats requiring them to reestablish dominant–subordinate relationships and territories. Chapman (1962) showed that coho fry moving downstream (nomads) into territories of a resident population of coho were dominated by the resident fish, resulting in their further downstream displacement. The implications of such movements are that the displaced fish may only be able to settle in unfavorable habitats where growth and survival are reduced. The possibilities we outlined indicate that exposure of fish to short-term pulses of suspended sediment may be deleterious to their fitness. The accumulated effects of repeated disruption of the social organization of the fish, reduced feeding, and physiological stress may incur energetic costs that might otherwise have been allocated towards growth. Reduced growth rates have been documented in steelhead trout (*Salmo gairdneri*) and coho fry reared in turbid water (Sigler 1981). The production of fish of small size and weakened condition renders them susceptible to adverse biological and environmental factors.

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Effects of Density and Microdetritus Enrichment on the Growth of Chironomid Larvae in a Small Pond

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Rasmussen, J. B. 1985. Effects of density and microdetritus enrichment on the growth of chironomid larvae in a small pond. Can. J. Fish. Aquat. Sci. 42: 1418–1422.

Densities, species-ratios, and food regimes of chironomid larvae were manipulated in enclosures placed in a small prairie pond, and effects of these treatments on the growth rates of larvae of *Chironomus riparius* and *Glyptotendipes paripes* were measured. These two species have tubiculous microphagous larvae that make up the majority of the benthos of the study pond. Intraspecific density effects strongly reduced growth rates for both species; however, interspecific effects were weak and sometimes not statistically significant. *Chironomus riparius* responded to the addition of sedimented microdetritus with a substantial increase in its growth rate, whereas *G. paripes* showed no response. The results indicate that the larvae of the two species can coexist stably at densities high enough to result in substantial intraspecies competition, and that differences associated with their mode of feeding contribute to resource partitioning. A sharp reduction in chironomid larval densities resulting from poor weather during the emergence period showed that the abiotic environment can override density effects in determining year-to-year abundance of these larvae.

Dans des enclos situés dans un petit étang de la prairie canadienne, on a fait varier la densité, le rapport des espèces et les régimes alimentaires de larves de chironomidés; on a aussi quantifié l'incidence de ces variations sur les taux de croissance des larves de *Chironomus riparius* et de *Glyptotendipes paripes*. Celles-ci, microphages et tubicoles, constituaient la plus grande partie du benthos de l'étang expérimental. Les effets intraspécifiques de la densité ont fortement réduit les taux de croissance chez les deux espèces; toutefois, les effets interspécifiques ont été faibles et parfois statistiquement non significatifs. Suite à l'apport de microdétritus sédimentaire, on a observé un accroissement important du taux de croissance chez *C. riparius* alors que *G. paripes* n'a pas réagi. Les résultats portent à croire que les larves de ces deux espèces peuvent coexister de manière stable à des densités assez élevées pour entraîner une forte compétition intraspécifique et que les différences entre leur mode d'alimentation contribuent au compartimentage des ressources. Une baisse rapide des densités de larves causée par le mauvais temps pendant la période d'émergence a révélé que l'environnement abiotique peut avoir plus d'importance que les effets de la densité dans la détermination de l'abondance de ces larves d'une année à l'autre.

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Chironomus riparius Meigen and *Glyptotendipes paripes* (Edwards) frequently coexist in small prairie ponds (Driver 1977; Rasmussen 1984a). Larval densities in these ponds can be extremely high and species richness very low. This suggests that competition might be important, and that partitioning of at least some resources by the two species would be required for prolonged coexistence. A few studies of competition between chironomid larvae have been carried out (Kajak 1963; Kajak and Dusoge 1967; Cantrell and McLachlan 1977; Wiley 1981); however, these have examined neither intraspecific and interspecific density effects on larval growth, nor have they attempted food resource manipulations.

By manipulating chironomid densities within enclosures, the following questions were addressed: (1) What is the relationship between larval density and growth rate, and does competition substantially reduce growth rates at the ambient densities? (2) What are the relative strengths of interspecific versus intraspecific density effects? (3) How is the growth of the two chironomid species and its relationship to density affected by enrichment with sedimented microdetritus?

Methods

Study Area and Its Biota

Stephenson Pond (<2 m deep and 2 ha in area; 114°16′W; 51°9′N), a hypereutrophic pond near Calgary, Alta., featured total densities of *C. riparius* and *G. paripes* in excess of 25 000/m^2. These two species, in addition to the predatory leech *Nephelopsis obscura* Verrill, made up all but approximately 2% of the benthic biomass of the pond. Limnological information on Stephenson Pond, the life cycles and production ecology of the chironomid larvae, and the effect of *Nephelopsis* on the chironomid growth and competition were described by Rasmussen (1983, 1984b). The experiments described here were all

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TABLE 1. Summary of experiments and their rationale (CR = *C. riparius*; GP = *G. paripes*).

Experiment	Purpose	Species ratio	Enclosure (m^2)	Density treatments	
				Normal food regime	Enriched food regime
1 (Fig. 2a)	Analysis of growth vs. density for normal vs. enriched food regimes	9:1 CR:GP	0.35	6 densities from 1 to 4.2 larvae/cm^2	5 densities from 1 to 3.25 larvae/cm^2
2 (Fig. 2b)	Analysis of growth vs. density (normal food regime)	1:9 CR:GP	0.35	7 densities from 0.2 to 2.9 larvae/cm^2	—
3 (Fig. 2c)	Analysis of growth vs. density for enriched vs. normal food regimes	1:9 CR:GP	0.07	4 densities from 0.3 to 2.5 larvae/cm^2	4 densities from 0.5 to 2.6 larvae/cm^2

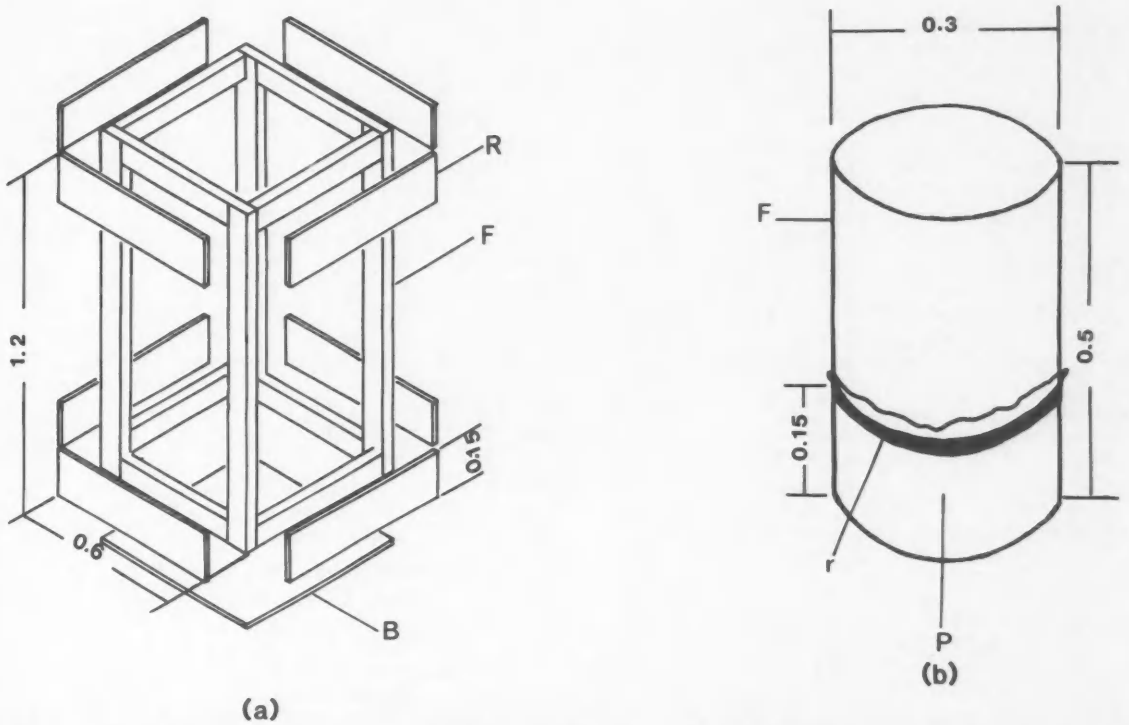

FIG. 1. Enclosures used in field experiments. (a) 0.35-m^2 enclosure (exploded view); the sides of the frame (F) were covered with 0.25-mm-mesh cloth and steel chicken wire. The structure was supported by rails (R) and a bottom (B). (b) 0.07-m^2 enclosure (lateral view); the frame (F), a cylinder made of 5-cm-mesh galvanized steel, was covered with 0.25-mm-mesh cloth. Thick polyethylene (P) covered the bottom and was held in place by a rubber band (r). All measurements in metres.

carried out in September 1980 and 1981 on growing fourth instar chironomid larvae in the absence of leeches. Last instars were used because earlier instars are of short duration and are much more sensitive to sieving and manipulation. Experiments were performed in September because reproductive activities (pupation, emergence, and oviposition) had completely ceased, thereby stabilizing the test populations. Temperatures during the experimental period ranged from 8 to 13°C in both years, which is sufficiently high to permit the larvae to feed actively and grow rapidly.

Density Manipulation

Mixtures of *C. riparius* and *G. paripes* were established within enclosures (Table 1; Fig. 1) at different densities, and growth of each species at each density was measured by the change in weight over the 25-d experimental period. Density manipulation was carried out on two different larval mixtures, a 9:1 CR:GP mixture (1980) and a 1:9 CR:GP mixture (1981). These two species ratios were chosen because they were strongly contrasting ratios, and because natural larval popula-

tions featuring both these ratios were available within the study area, thus eliminating the need for individual enumeration and handling of larvae. For both species ratios, density effects on the most abundant species will be predominately intraspecific, whereas interspecific effects would be mainly expressed by the density effects on the least abundant species.

Installation and Sampling of Enclosures

The experimental manipulations were carried out within the enclosures shown in Fig. 1. Due to lower larval densities in the study area during 1981, more time was required to set up the experiments. Thus, to perform the 1:9 CR:GP food addition experiments within the same time frame with the remainder of the 1:9 CR:GP experiments, the smaller more rapidly set-up 0.07-m^2 enclosures were used (Table 1). Water and microplankton could circulate across the 0.25-mm mesh covering the enclosures, but neither chironomid larvae nor leeches could enter or leave. A 10- to 15-cm layer of sieved mud was added to the bottom of the enclosures, and the desired larval mixture and density for each treatment was added and allowed to colonize the sediment. To avoid excessive handling of larvae, Ekman grabs taken from an area of known larval density were sieved, leeches were removed, and the sieve residue containing the chironomid larvae was added to the enclosure. The enclosures were placed in water of 1 m depth with the bottoms pushed 10–15 cm into the mud. After the mud had settled, sufficient pond water was added to bring the inside concentration of *Aphanizomenon* colonies up to that of the ambient water. The enclosures were allowed to stand undisturbed for 3 d before initial sampling. Mean densities and mean wet weight of chironomid larvae inside the enclosures were estimated by removing six replicate 20-cm^2 cores at the beginning and end of the experimental period.

The vertical profile of dissolved oxygen and the chlorophyll *a* concentration of the water column were measured both inside the enclosures and in the ambient water. Dissolved oxygen was measured with a YSI DO probe, and chlorophyll *a* was measured by the Scor/UNESCO spectrophotometric technique described by Strickland and Parsons (1972).

Calculations and Statistical Analyses

The treatment density for each enclosure was calculated as the average of the initial and final densities (12 cores); no significant differences between initial and final density estimates were detected within any of the enclosures, or across all of them (paired *t*-test), indicating that mortality in these experiments was negligible. Within each experiment, no significant between-enclosure differences in starting weights of larvae were found; therefore, the initial weights of each species in each experiment were taken as the pooled mean over all enclosures comprising the experiment. This greatly reduced the variance associated with both the starting weight and the weight change throughout the experiment. At the termination of each experiment, one or two Ekman grabs were taken from each enclosure after the core samples had been removed. This provided a larger sample of larvae for weighing, which increased the precision of the final larval weights. This was especially important in estimating the growth increment of the least abundant species in the low-density treatments.

The growth rates of each species of chironomid were estimated as the change in mean wet weight divided by the duration of the experiment. Absolute growth (rather than any of its commonly used transformations such as weight-specific growth rate or exponential growth rate (see Winberg 1971)) was used because (1) in all of the experiments, larvae of the two species were of nearly equal weight at the outset, and (2) Rasmussen (1983) had found that growth rates of fourth instars of both species during August and September were uncorrelated with larval weight. Thus, there was no need to introduce complexity by transforming growth data to compensate for differences in initial larval weight. Mean weight was obtained for each species in each sample by collectively weighing the blotted larvae (nearest 0.01 mg) and dividing by the number present. Model 1 least squares linear regression analysis together with analysis of covariance (Zar 1974) were used to analyze the effects of larval density and organic enrichment on the weight gained by larvae during the experiments. The strength of the density effect was measured by the magnitude of the negative slope (growth increment vs. density), and slopes and intercepts were compared by a *t*-test as described by Zar (1974). Statistical significance refers to $p < 0.05$ except when otherwise stated.

Enrichment with Organic Detritus

The detritus added to the organically enriched enclosures was prepared by adding finely ground Tetra-Min fish food to sieved pond mud (1 g of Tetra-Min to 100 mL of fine mud). Biever (1971) and Credland (1973) have shown that Tetra-Min is an excellent food source for many species of chironomid larvae in laboratory cultures. After being thoroughly stirred, the mixture was allowed to stand and decompose for 3 d with periodic stirring. By this time the fine Tetra-Min and mud particles were found to be coated with microflora when examined microscopically, providing a food source qualitatively similar to the fine detritus normally consumed by deposit-feeding benthic chironomid larvae. Each week, 200 mL of this mixture was added to each of the treated 0.35-m^2 enclosures, and 40 mL of the mixture was added to the 0.07-m^2 enclosures.

Results

Effect of Density and Food Regime on Larval Growth

In all of the experiments, significant negative effects of density on growth rates of one or both species were recorded. In the 9:1 CR:GP experiment (Fig. 2a; Table 2) the effect of density on the growth of *C. riparius* was highly significant both with ($p < 0.01$) and without ($p < 0.005$) organic enrichment; furthermore, the slope of the growth rate – density relationship was significantly steeper for *C. riparius* than for *G. paripes* under both food regimes. In the 1:9 CR:GP experiments (Fig. 2b and 2c; Table 2) the effect of density on the growth rate of *G. paripes* was highly significant under normal ($p < 0.001$) and enriched ($p < 0.01$) food regimes, and significantly stronger ($p < 0.01$) than the effect on *C. riparius* under both food regimes. Thus, the most abundant species always experienced much stronger retardation of growth by elevated density than did the least abundant species, indicating that intraspecific density effects were much stronger than interspecific effects. In fact, in three of five experiments, density effects on the growth of the least abundant species were not significant at $p < 0.05$ (Table 2).

Organic enrichment with microdetritus resulted in significantly enhanced growth of *C. riparius* (*t*-test comparison of zero-density intercepts). For the 9:1 CR:GP mixtures, enrichment resulted in significantly increased growth rate of *C.*

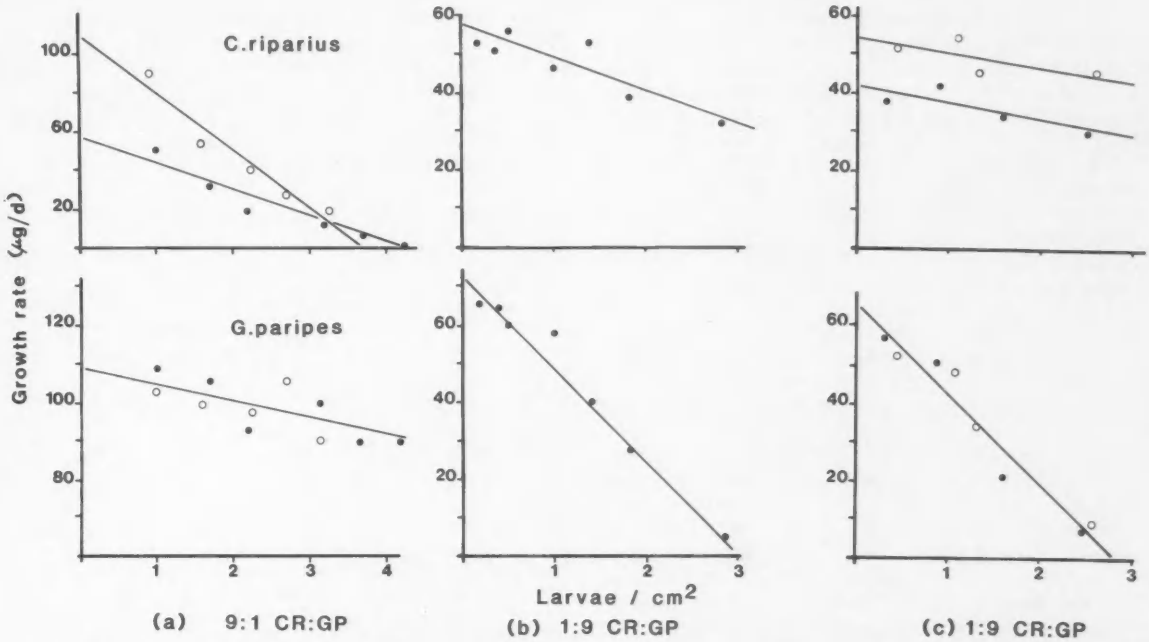

FIG. 2. Effect of total larval density on growth rates of *C. riparius* and *G. paripes* for larval species ratios (a) 9:1 CR:GP and (b and c) 1:9 CR:GP. Lines were fitted by linear least squares linear regression (see Table 2 for regression statistics). Closed circles = normal food regime; open circles = enriched food regime.

TABLE 2. Regression statistics for enclosure experiments (Fig. 2) (CR = *C. riparius*; GP = *G. paripes*).

Larval mixture	Food treatment	Figure	Species	Initial weight (mg)	Equation $G = f(N)$[a]	p	r^2
9:1 CR:GP	Normal	2a	*C. riparius*	3.41	$G = 57.5(\pm5.3) - 13.6(\pm1.83)N$	<0.005	0.93
			G. paripes	4.50	$G = 112.0(\pm6.6) - 5.2(\pm1.70)N$	<0.05	0.69
	Enriched	2a	*C. riparius*	3.43	$G = 108.0(\pm10.3) - 29.0(\pm4.5)N$	<0.01	0.92
			G. paripes	4.55	$G = 109.8(\pm5.8) - 4.42(\pm1.80)N$	<0.10	0.60
1:9 CR:GP	Normal	2b	*C. riparius*	2.98	$G = 57.8(\pm3.5) - 8.28(\pm2.34)N$	<0.05	0.71
			G. paripes	3.09	$G = 72.4(\pm3.4) - 24.4(\pm1.74)N$	<0.001	0.96
		2c	*C. riparius*	3.01	$G = 42.6(\pm3.9) - 4.55(\pm2.46)N$	<0.10	0.63
			G. paripes	3.11	$G = 66.3(\pm7.4) - 24.1(\pm4.72)N$	<0.01	0.92
		2c	*C. riparius*	3.04	$G = 54.5(\pm3.9) - 3.77(\pm2.50)N$	<0.10	0.53
			G. paripes	3.14	$G = 66.2(\pm5.5) - 21.6(\pm3.48)N$	<0.01	0.95

[a] G = growth rate expressed (µg/d); N = density (larvae/cm^2) (both species combined).

riparius for densities <2.8/cm^2. The effect of enrichment was less pronounced at high densities, indicating that interference effects were likely overriding food effects at the highest densities. *Chironomus riparius* also responded strongly to enrichment when it was the least abundant species (1:9 CR:GP, Fig. 2c; Table 2). In contrast, the enrichment had no effect on *G. paripes* in any of the experiments.

Enclosure Regime

Dissolved oxygen concentrations in the water adjacent to the enclosures were significantly higher than inside; however, inside levels were well in excess of the 10% saturation level. Berg et al. (1962) and Berg and Jonasson (1965) found that above 10% dissolved oxygen saturation, the oxygen consumption of *Chironomus anthracinus* was independent of the dissolved oxygen concentration of the water. Total chlorophyll *a* concentrations inside and outside the enclosures were not significantly different. In control enclosures, where the benthic community was identical to the ambient community, both species of chironomid larvae exhibited growth rates nearly identical to growth rates determined for larvae outside the enclosures (Rasmussen 1983). Furthermore, mortality of larvae in the enclosures was not significantly different from zero, another indication that the enclosure regime had little adverse effect on the experimental animals.

Discussion

The ambient chironomid density at the study site during 1980 ranged from 6.1 larvae/cm^2 in June (second instar larvae) to

2.2/cm^2 in September–October (fourth instars) (Rasmussen 1984b). Throughout this period about 90% of the larvae were *C. riparius* and about 10% were *G. paripes*. The density manipulation experiments showed that in the September fourth instar population, the growth rate of *C. riparius* was being retarded by density effects and that its growth rate was likely around one half of its potential rate, as estimated by the zero-density intercepts of the regressions shown in Fig. 2 and Table 2. On the other hand, the growth of *G. paripes* was not significantly different from its intercept value at the prevailing densities. Rasmussen (1983) showed that the main source of mortality affecting the larval chironomid population was predation by *Nephelopsis* leeches, and that without this cropping, intraspecific density effects could have nearly terminated the growth of *C. riparius* early in its fourth instar. Thus, it appears that the larval density, and factors that affect it, can strongly affect the rate of growth and development of chironomid larvae, and that the number of generations produced in one season may be partly determined by density effects. In fact, in two other studies of *C. riparius*, total densities of fourth instar chironomid larvae were always much less than 1/cm^2 (Learner and Edwards 1966; Gower and Buckland 1978) and the species in these studies exhibited a multivoltine life cycle rather than the univoltine one found here. Jonasson and Thorhauge (1976) also suggested that density reduction by predators in littoral areas contributes to higher population turnover and production.

The 9:1 CR:GP experiments (Fig. 2; Table 2) also showed that even if larval densities had been high enough to prevent growth of *C. riparius* completely, *G. paripes* would have been at most only slightly affected, and for the reversed species ratio (1:9 CR:GP), *C. riparius* would have been only slightly affected by high densities. Thus, it appears that larval competition between these species could not be expected to result in the elimination of either species from the population, and that the two species should be capable of stable coexistence even under densities high enough to greatly reduce growth.

This outcome indicates that some resources are being effectively partitioned between the larvae of the two species. Spatially, the larvae showed no apparent segregation; in fact, their abundance in core samples (both inside and outside enclosures) was positively correlated ($r = 0.55$; $p < 0.001$). The response of the two species to the addition of microdetritus to the sediment surface, however, showed that *C. riparius* could utilize this resource, whereas *G. paripes* could not. This is consistent with results of gut content analyses (Rasmussen 1984a) and observations of behavior and tube morphology (Rasmussen 1984b), which indicate that *C. riparius* feeds by ingesting fine particles at the sediment surface, whereas *G. paripes* filters fine particles from the water column while remaining inside its tubes. Besides reducing exploitative competition for food, the different feeding mechanisms probably result in reduced interspecific interference effects, since the habitat is being utilized differently by the two species.

The experiments described clearly indicate that density can exert strong effects on the growth of chironomid larvae in Stephenson Pond. However, Rasmussen (1983) showed that year-to-year variation in larval densities could be greatly affected by spring weather conditions, which in 1981 were so severe as to greatly reduce larval recruitment of chironomids to the pond. In fact, over the 5-yr period during which fall densities of chironomid larvae in the pond were monitored (Rasmussen 1983), densities were high (>1.5 larvae/m^2) in only two of the years (1979 and 1980) and very low (<0.5/m^2) in 1981 and 1982. Hence, density effects on growth would be expected to be quite variable from year to year in this system.

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Catch-Per-Unit-Effort: A Statistical Model for Pacific Halibut (*Hippoglossus stenolepis*)

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The empirical distributions of catch-per-unit-effort (CPUE) of Pacific halibut (*Hippoglossus stenolepis*) were positively skewed and could be modeled by a general family of distributions involving power transformations. The root normal distribution, a special case from the family, was found to be a convenient model for the data. Measures of the central tendency and variance of the distribution were derived. Estimators of the central tendency based on increasing variance of a function of catch as a function of effort were most appropriate for the data. A median-based estimator performed better than a mean-based estimator. Thus, statistical modeling of CPUE provided a tool for constructing a better estimator of an abundance index.

Les distributions empiriques des prises par unité d'effort (PPUE) du flétan du Pacifique (*Hippoglossus stenolepis*) étaient désaxées vers la droite et pouvaient être modélisées par une famille générale de distributions comprenant des transformations de puissance. La distribution normale des racines, un cas spécial appartenant à cette famille, s'est révélée un modèle pratique pour la présentation des données. La tendance centrale et la variance de la distribution ont aussi été calculées. Des estimateurs de la tendance centrale basés sur la variance croissante d'une fonction décrivant les prises par rapport à l'effort se sont révélés les plus appropriés. Un estimateur basé sur la médiane s'est révélé meilleur qu'un estimateur basé sur la moyenne. La modélisation statistique des PPUE a ainsi fourni un outil pour le calcul d'un meilleur estimateur de l'indice d'abondance.

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Catch-per-unit-effort (CPUE) is a direct, easily calculated statistic from a fishery, frequently used as an index of abundance of a fish population. The mathematical background of CPUE has been well studied, derived from assumptions about the catchability of fish from a particular type of gear (Beverton and Holt 1957; Gulland 1983). The statistical approach to modeling CPUE has not been pursued to the same extent but could provide valuable insights into the nature of the process of catching fish. Further, an understanding of the underlying statistical distribution for CPUE is necessary to undertake appropriate estimation of the mean, variance, and other parameters of the distribution and to make valid inferences from the data. An ideal estimator would accurately describe the central tendency of the distribution, be robust to the influence of data outliers, and have low sampling variability.

For some fisheries, the distribution of CPUE has been studied. Smith (1980) recommended a jackknife estimator to remove bias and to obtain a valid variance estimate. Gavaris (1980) suggested that CPUE can be modeled using a lognormal distribution. Bannerot and Austin (1983) modeled CPUE with a negative binomial distribution and recommended an estimator using the proportion of zero values in the data. Pennington (1983) derived a CPUE distribution that was modeled as a mixture of a mode at the zero value and a standard distribution (e.g. the lognormal) for the rest of the data. He developed appropriate estimators for such a distribution. Quinn et al. (1982) used ratio estimators to evaluate two methods of combining CPUE across geographic region, based on empirical evidence that the variance of catch was proportional to effort.

My objective was to investigate the statistical distribution for CPUE of Pacific halibut and to construct appropriate estimators of parameters of the distribution. The distribution of the data is modeled with a general family of distributions involving power transformations, and maximum likelihood estimation is used to select a specific distribution from the family. Median-based estimators are constructed to meet the concerns of accuracy and precision mentioned above, especially for skewed distributions, and are contrasted with commonly used mean-based estimators. Finally, the distributions that occur from the statistical modeling of CPUE in fisheries are hypothesized to be related to the degree of aggregation in the catching process and the amount of knowledge about the population.

Empirical Distribution of CPUE

The International Pacific Halibut Commission (IPHC) collects daily catch and fishing effort data from several fishing vessels each year. Catch is recorded in weight of fish in pounds (2.2 lb. = 1 kg), and a unit of effort is a "skate," 1800 ft (550 m) of longline gear with 100 equally spaced hooks. It can be assumed that fishing effort is measured without error, since fishermen are well aware of the amount of gear they use. The

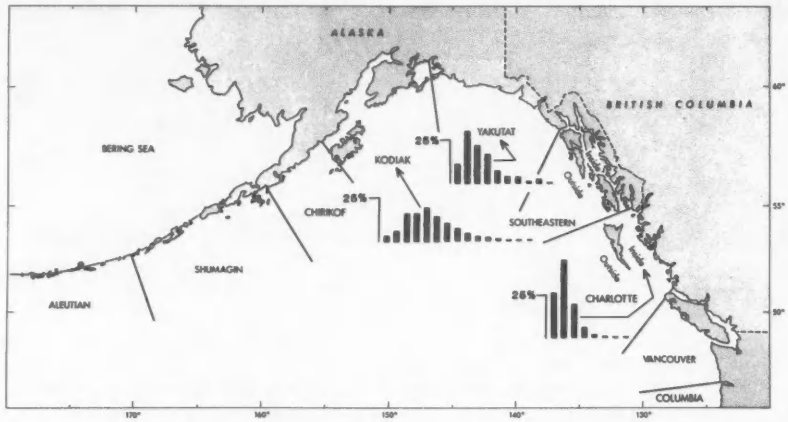

FIG. 1. Region designations used by IPHC and histograms (percent frequency) of CPUE (40 pounds/skate intervals starting with (0–40), (40–80), etc.) for regions Charlotte (inside), Yakutat, and Kodiak.

details of collecting the information and regional estimates since 1929 are given by Myhre et al. (1977). Standardization of effort data has been well studied by IPHC, and the major factor is the spacing of hooks.

CPUE data from three regions in 1982 had large numbers of daily observations for analysis of distribution. Region designations used by IPHC and histograms of CPUE for the three regions are shown in Fig. 1. Although the three empirical distributions have different central tendencies, their shapes are similarly positively skewed. Such distributions may arise when a few fishermen catch a high proportion of the catch or some type of aggregation exists in the catching process.

Two transformations (logarithmic, square root) were applied to the data to attempt a normal approximation. The logarithmic transformation made the empirical distribution negatively skewed, but the square root transformation made the data appear normally distributed. Tests of normality with the Anderson–Darling EDF statistic (Stephens 1974) confirmed that the square root transformation approximated normality better than the raw data or a logarithmic transformation of the data for the three regions (rows labeled "All" in Table 1).

The data were broken into 10 classes based on the amount of effort per day to determine the distribution of the data on a finer scale. Effort per day may be one measure of fishing power of a vessel, because small vessels may not be able to fish as much as large vessels. Tests were performed for each class on the raw CPUE data, square root, and logarithm of the data; results from the tests for the three regions are shown in Table 1. Rejection at $\alpha = 0.05$ of the hypothesis of normality occurred 9 out of 30 times for the square root transformation, 14 out of 30 times for the logarithmic distribution, and 19 out of 30 times for the raw data. The test statistic was lowest among classes 16 times for the square root transformation, 12 times for the logarithmic transformation, and 2 times for the raw data. Hence, transformation of the data made the data more normally distributed, with a slight nod to the square root over the logarithmic transformation, although some discrepancies from exact normality were still present.

The family of power transformations of a random variable Y, denoted by Y^{λ}, can be used to determine what transformation of the data can be modeled by a normal distribution. This family covers a wide range of transformations, including logarithmic

TABLE 1. Tests of normality using the Anderson–Darling statistic (Stephens 1974) of the raw CPUE data, square root of the data, and logarithm of the data within effort class for three regions, 1982 (*significant at $\alpha = 0.05$; **significant at $\alpha = 0.01$).

Effort class (skates/day)	n	Test statistic		
		CPUE	$CPUE^{1/2}$	ln (CPUE)
Charlotte (inside)				
1–10	36	1.0492*	0.3226	0.7584
11–15	52	1.8210**	0.5389	1.2031**
16–20	94	1.6695**	0.4651	1.4344**
21–25	88	0.7912*	0.4292	2.4940**
26–30	126	3.1050**	0.6048	1.3218**
31–35	59	0.5669	0.1780	0.5856
36–40	56	5.4707**	3.0399**	2.1153**
41–45	35	0.1951	0.4915	1.7601**
46–50	16	0.6195	0.3486	0.2611
⩾51	21	0.2950	0.2407	0.7832
All	583	10.1750**	1.2011**	8.3600**
Yakutat				
1–10	15	0.8546*	0.3062	0.3709
11–15	20	1.4345**	1.1085**	1.1969**
16–20	13	0.9367*	0.5528	0.3726
21–25	18	0.3687	0.3679	0.7996*
26–30	23	1.9526**	1.0659*	0.4657
31–35	19	1.1540**	0.5383	0.2959
36–40	23	1.1528**	1.2680**	2.1351**
41–45	18	1.1800**	0.8917*	1.2425**
46–50	14	1.3394**	1.1238**	0.9918*
⩾51	38	1.7572**	0.6847	0.2650
All	201	6.0564**	1.0305*	1.2533**
Kodiak				
1–10	5	0.3702	0.3209	0.2726
11–15	7	0.5044	0.4346	0.3843
16–20	21	1.2402**	1.1180**	1.8517**
21–25	11	0.3681	0.3616	0.4331
26–30	26	0.7658	0.4364	0.6793
31–35	11	0.5427	0.4636	0.4292
36–40	37	2.0659**	1.1635**	0.9052*
41–45	26	0.6193	0.3515	0.4518
46–50	69	2.6679**	1.2641**	0.6672
⩾51	173	1.6189**	0.6461	1.2471**
All	386	3.7489**	0.5807	5.8872**

TABLE 2. Maximum likelihood estimation of transformation for 1982 regional CPUE data: sample size (n), estimate ($\hat{\lambda}$), 95% confidence interval (λ_l, λ_u), and log likelihoods (L) at $\lambda = \hat{\lambda}$, 0 (log transformation), 0.5 (square root transformation), and 1 (untransformed data).

Region	n	Maximum likelihood estimate $\hat{\lambda}$	(λ_l, λ_u)		ln $L(\hat{\lambda})$	Logarithmic ln $L(0)$	Square root ln $L(0.5)$	Untransformed ln $L(1)$
Charlotte (outside)	86	0.9	0.6	1.3	−290	−306	−292	−290
Charlotte (inside)	583	0.4	0.3	0.5	−2084	−2125	−2087	−2161
SE AK (inside)	83	−0.1	−0.4	0.2	−366	−367	−373	−388
Yakutat	201	0.2	0.1	0.4	−833	−838	−837	−871
Kodiak	386	0.5	0.4	0.6	−1749	−1789	−1749	−1775
Chirkof	128	0.2	−0.2	0.6	−570	−571	−572	−580
Shumagin	196	0.3	0.1	0.4	−824	−831	−828	−870

($\lambda = 0$), square root ($\lambda = \frac{1}{2}$), and untransformed ($\lambda = 1$). Maximum likelihood estimation of λ provides a quantitative tool for choosing the appropriate transformation (Box and Cox 1964; Draper and Smith 1981, p. 225–235; Carroll and Ruppert 1984). Maximum likelihood estimates of λ for 7 regions are shown in Table 2, along with 95% confidence intervals, and log likelihoods at various λ values. Estimates of λ range from −0.1 to 0.9, with fairly tight confidence intervals for each region. The log likelihood for the square root transformation generally is higher than those for either the logarithmic or untransformed, and not much lower than the maximum likelihood. The most likely value for λ across regions is 0.4; five of seven confidence intervals contained this value. Only three intervals contained $\frac{1}{2}$, but the practical difference between $\frac{1}{2}$ and 0.4 was judged to be insignificant. Thus, a square root transformation is a convenient transformation of the CPUE data in order to approximate normality, providing a middle ground between the raw data and a logarithmic transformation. For each transformation, a corresponding statistical distribution exists, including lognormal ($\lambda = 0$), root normal ($\lambda = \frac{1}{2}$), and normal ($\lambda = 1$).

Modeling CPUE with the Root Normal Distribution

The classical deterministic relationship between CPUE and abundance (N) is CPUE = qN, where q is the catchability coefficient. The stochastic treatment of this relationship imposes a statistical distribution upon the variable CPUE where the central tendency of the distribution is parameterized by qN. Several measures of central tendency can be considered.

To derive relationships from the root normal distribution, let Y be the random variable representing CPUE and let X be the square root of Y, i.e. $X = Y^{1/2}$. It is assumed that X is normally distributed with mean μ and variance σ^2, denoted $N(\mu, \sigma^2)$. The derivation of the root normal distribution of Y and its parameters is given in the Appendix. The traditional parameters are the mean and variance, given by

$$(1) \quad E(Y) = \mu^2 + \sigma^2$$

$$(2) \quad \text{Var}(Y) = 4\mu^2\sigma^2 + 2\sigma^4.$$

The median can be approximated by

$$(3) \quad m(Y) = \mu^2$$

if $\mu > \sigma$. The mode is an intractible function, but can be approximated by $[\mu + (\mu^2 - 4\sigma^2)^{1/2}]^2/4$.

From the above equations, the mode is less than the median, which in turn is less than the mean. On theoretical grounds, the median would be preferred to the mean and the mode. The median is the result of placing equal probability on either side; for the mean and mode, the probability on one side depends on the degree of skewness.

Parameter Estimation

Suppose that an independent random sample of n observations $\{y_i\}$ is taken from a root normal distribution. Taking advantage of the fact that $Y^{1/2}$ is $N(\mu, \sigma^2)$, the usual unbiased estimates of μ and σ^2 are

$$(4) \quad \hat{\mu}_1 = \Sigma y_i^{1/2}/n$$

$$(5) \quad \hat{\sigma}_1^2 = \Sigma(y_i^{1/2} - \hat{\mu}_1)^2/(n-1)$$

with respective variances

$$(6) \quad \text{Var}(\hat{\mu}_1) = \sigma^2/n$$

$$(7) \quad \text{Var}(\hat{\sigma}_1^2) = 2\sigma^4/(n-1).$$

Either the mean or median of the root normal distribution may be considered a valid measure of central tendency. The mode is not considered, because it is a complicated, unwieldy function and the statistical properties of the mode are in general less noteworthy. If the statistical distribution is not investigated, most applications use the sample mean $\bar{y}$ as the measure of central tendency. The sample mean is an unbiased estimator of $E(Y) = \mu^2 + \sigma^2$. Its variance from (2) is

$$(8) \quad \text{Var}(\bar{y}) = \text{Var}(Y)/n = (2\sigma^4 + 4\sigma^2\mu^2)/n.$$

An estimator of the median μ^2 follows naturally from (3) and (4), given by

$$(9) \quad \hat{m}_1 = \hat{\mu}_1^2 = (\Sigma y_i^{1/2}/n)^2.$$

Because $\hat{\mu}_1$ is $N(\mu, \sigma^2/n)$, $\hat{\mu}_1^2$ has the corresponding root normal distribution with mean from (1)

$$(10) \quad E(\hat{m}_1) = \mu^2 + \sigma^2/n$$

and variance from (2)

(11) $\text{Var}(\hat{m}_1) = 4\sigma^2\mu^2/n + 2\sigma^4/n^2$.

Note that the second term in (11) is negligible for large n. Comparing (11) and (8) shows that the variance of the estimated mean is larger than the variance of the estimated median.

From (10), the estimated median is a biased estimator of the true median. However, because the estimator $\hat{m}_1$ has a root normal distribution, the median of $\hat{m}_1$ is the desired measure of its central tendency. Using (3), the median of $\hat{m}_1$ is

$$m(\hat{m}_1) = \mu^2,$$

that is, $\hat{m}_1$ is median-unbiased (McCaughran 1981). This property and the lower variance of the estimator $\hat{m}_1$ compared with $\bar{y}$ suggest that the median is the desired estimator of central tendency of the root normal distribution. The estimator $\hat{m}_1$ is used instead of the sample median in order to study methods of combining data in the next section. The sample median would be preferable in terms of robustness to $\hat{m}_1$, but $\hat{m}_1$ is more malleable for computation and weighting.

One referee of this paper noted an interesting alternative development of estimators of the root normal distribution. By reparameterizing the root normal distribution in terms of a location parameter $\tau = \mu^2$ and a scale parameter $\nu^2 = 4\mu^2\sigma^2$, it can be shown that the method of maximum likelihood can be used to obtain estimates of τ and ν^2 (corrected for bias), which are analogous to those obtained above. When σ is much less than μ, then it can be shown that the mean and median of the root normal distribution are equal to τ and the variance is equal to ν^2. The approach presented above, however, only requires the simple inequality $\sigma < \mu$ and hence is more general.

The use of an estimator from an incorrect distribution may occur in practice due to variability among regions, years, or vessels. To study this effect, data from the 10 effort classes of the three regions were again used. For each class, the sample mean (associated with the normal distribution), the estimator $\hat{m}_1$ (associated with the root normal distribution), and the estimated median $\exp[\Sigma \ln(y_i)/n]$ (associated with the lognormal distribution) were computed. Pairwise Spearman correlation coefficients of the estimators using the 30 independent observations were all above 0.95 and highly significant ($P < 0.001$). Thus, the use of a single estimator of CPUE may be warranted, even when the data differ from the hypothesized distribution.

Combining Data

A disadvantage of the estimators $\hat{m}_1$ and $\bar{y}$ in the previous section is that they treat each daily observation with equal weight. An increase in the amount of fishing effort should increase the precision and more weight should be given to observations with more fishing effort. Mathematically,

(12) $C^{1/2} = \mu(E)E^{1/2} + \xi$

where C is daily catch, E is daily effort, CPUE $= C/E$, and ξ is assumed to be distributed $N(0, \sigma^2(E))$. The mean and variance are both written as functions of effort pending examination of the data.

If the central tendency of the distribution of $(\text{CPUE}^{1/2})$ is not a function of effort ($\mu(E) = \mu$), then three estimators of μ can be considered based on the variance structure of the data.

[1] If $\sigma^2(E) = E\sigma^2$, the estimator $\hat{\mu}_1$ in equation (4) results.

[2] If $\sigma^2(E) = E^{1/2}\sigma^2$, then one obtains the ratio estimator

(13) $\hat{\mu}_2 = \Sigma C^{1/2}/\Sigma E^{1/2}$.

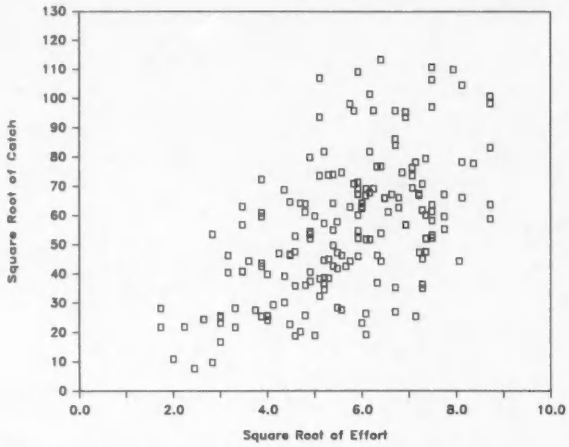

FIG. 2. $C^{1/2}$ versus $E^{1/2}$ from daily observations in the Yakutat region, 1982.

[3] If $\sigma^2(E) = \sigma^2$, then one obtains the estimator

$$\hat{\mu}_3 = \Sigma(CE)^{1/2}/\Sigma E.$$

Note that these variance functions are for $C^{1/2}$, not $\text{CPUE}^{1/2}$. The corresponding variances for $\text{CPUE}^{1/2}$ are [1] σ^2, [2] $E^{-1/2}\sigma^2$, and [3] $E^{-1}\sigma^2$. Hence, for variance model [2], the variance of $C^{1/2}$ is proportional to $E^{1/2}$, but the variance of $\text{CPUE}^{1/2}$ is proportional to the inverse of $E^{1/2}$, indicating that $\text{CPUE}^{1/2}$ is more precise with increasing effort.

These estimators are best linear unbiased estimators for the given variance function. Other estimators may be appropriate if a different variance function is used or if a multiplicative error structure is assumed. If $\mu(E)$ is not constant, then a stratified estimator should be considered.

Parameters for these models may be estimated with least squares or maximum likelihood, which result in equivalent estimators with underlying normality. Graphic depiction of the daily catch data and analysis of residuals are often used to determine the appropriate error structure. However, the variability in daily CPUE data of Pacific halibut is too large to assess trends in either catch or variance; an example of $C^{1/2}$ versus $E^{1/2}$ from the Yakutat region is shown in Fig. 2.

An alternative procedure used data from the three regions broken into 10 effort classes, as before. The estimated mean and variance of $C^{1/2}$ were calculated from (4) and (5). This breakdown made it possible to perform linear regression analyses using $C^{1/2}$ and the sample variance of $C^{1/2}$ as the dependent variables, with $E^{1/2}$ or E for each class as the independent variable. These dependent variables were used instead of the CPUE variables, so that the independent and dependent variables would not both be functions of effort.

The variable $C^{1/2}$ was plotted versus $E^{1/2}$ of each effort class for the three regions in Fig. 3. The relationship between $C^{1/2}$ and $E^{1/2}$ appears to be linear. A linear relationship was superior to a constant or quadratic function in terms of minimum residual sum of squares (Table 3), suggesting that $\mu(E) = \mu$ in (12).

The sample variance of $C^{1/2}$ was plotted versus $E^{1/2}$ for the three regions in Fig. 4. For Charlotte and Yakutat, the variance appears to increase linearly with $E^{1/2}$, but for Kodiak there is too much variability to visually determine the appropriate relationship. In terms of minimum residual sum of squares, a linear

TABLE 3. Residual sum of squares (RSS) and parameter estimates for model fits of $C^{1/2}$ and variance of $C^{1/2}$ versus functions of effort for Charlotte, Yakutat, and Kodiak. Data are average $C^{1/2}$, estimated variance of $C^{1/2}$, and average $E^{1/2}$ as a function of 10 effort classes: (1, 10), (11, 15), (16, 20), (21, 25), (26, 30), (31, 35), (36, 40), (41, 45), (46, 50), (51+) skates.

	$C^{1/2} = \mu(E) + \xi$			Var $(C^{1/2}) = \sigma^2(E)$		
Region	Model	RSS	Estimate ± 1 SE	Model	RSS	Estimate ± 1 SE
Charlotte (inside)	μ	1267	42.1±3.8	$\sigma^2 E$	48 570	169±23
	$\mu E^{1/2}$	55	7.75±0.14	$\sigma^2 E^{1/2}$	13 830	31.8±2.2
	μE	948	1.20±0.09	σ^2	14 000	5.06±0.35
Yakutat	μ	1897	53.5±4.6	$\sigma^2 E$	140 400	323±40
	$\mu E^{1/2}$	210	9.90±0.27	$\sigma^2 E^{1/2}$	81 300	59.7±5.4
	μE	1906	1.54±0.13	σ^2	150 400	9.29±1.18
Kodiak	μ	6246	72.0±8.3	$\sigma^2 E$	270 900	469±55
	$\mu E^{1/2}$	479	13.6±0.4	$\sigma^2 E^{1/2}$	169 900	85.8±7.8
	μE	2238	2.12±0.14	σ^2	318 600	13.2±1.7

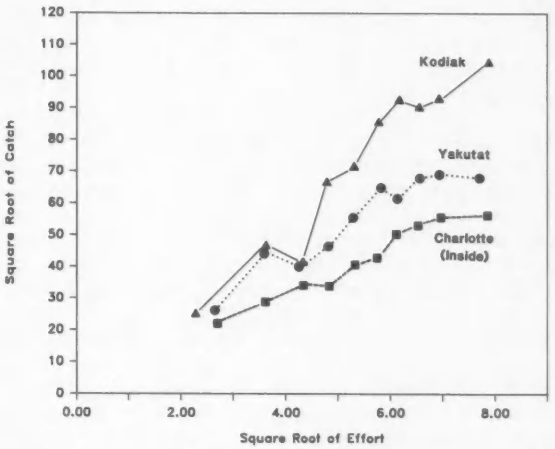

FIG. 3. $C^{1/2}$ versus $E^{1/2}$ broken into 10 effort classes for three regions, 1982.

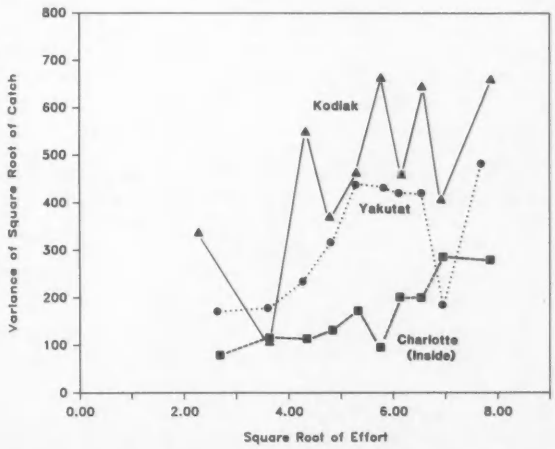

FIG. 4. Sample variance of $C^{1/2}$ versus $E^{1/2}$ broken into 10 effort classes for three regions, 1982.

relationship is best for all three regions (Table 3). In light of these results, variance model [2], which features a linear increase in $E^{1/2}$, is more appropriate than a constant or quadratic function. Thus, the estimator $\hat{\mu}_2$ in (13) is the appropriate estimator for CPUE data. It is not unbiased but consistent (Cochran 1963). The variance estimator of $\hat{\mu}_2$ comes from the theory of ratio estimation (Cochran 1963) and is given by

$$\hat{\sigma}_2^2 = \frac{n}{n-1} \Sigma (C^{1/2} - \hat{\mu}_2 E^{1/2})^2 / (\Sigma E^{1/2})^2.$$

An approximately median-unbiased estimator of the median from (9) is

(14) $\hat{m}_2 = \hat{\mu}_2^2$.

The estimated variance of $\hat{m}_2$ is

(15) $\hat{\text{Var}}(\hat{m}_2) = 4\hat{\sigma}_2^2 \hat{\mu}_2^2 + 2\hat{\sigma}_2^4$,

which follows from (11) replacing σ^2/n by $\hat{\sigma}_2^2$.

Comparison of Mean and Median CPUE

The estimator currently used by IPHC is the ratio estimator $\Sigma C/\Sigma E$, which was described by Myhre et al. (1977) and Quinn et al. (1982). This estimator is nearly unbiased for the mean of the distribution of CPUE, when the variance of catch increases linearly with effort (Quinn et al. 1982). In contrast, the estimator $\hat{m}_2$ in (14) is a nearly median-unbiased estimator of the median of the CPUE distribution, when the variance of the square root of catch increases linearly with the square root of effort.

The estimated mean and median and their standard errors (square root of the variance) are shown for three IPHC regions (Table 4). As expected for a positively skewed distribution, the mean is generally greater than the median. Also, the standard error of the mean is generally greater than the standard error of the median, confirming the theoretical result presented earlier. However, the difference between the mean and median and the difference in the two standard errors are relatively small, generally less than 10%. The mean and median values were computed for all 12 IPHC regions between 1972 and 1983 for American vessels and Canadian vessels. For both nations, median CPUE is a linear function of mean CPUE with slope of about 0.93. The Spearman correlations between the two are highly significant (American, $r = 0.997$, $P < 0.001$; Canadian,

TABLE 4. Comparison of mean and median CPUE (pounds per skate).

Year	Mean	SE	Median	SE
	Charlotte (inside) — Canadian vessels			
1972	71.8	1.4	65.2	1.3
1973	71.0	1.5	63.2	1.4
1974	66.7	1.9	62.9	1.9
1975	66.8	1.4	62.0	1.3
1976	54.6	1.1	49.8	1.0
1977	63.0	1.8	56.4	1.6
1978	59.2	1.6	53.9	1.5
1979	45.2	1.2	41.3	1.1
1980	68.7	2.1	60.6	1.7
1981	63.5	1.9	54.6	1.6
1982	65.3	1.7	60.0	1.5
1983	84.1	2.7	77.7	2.4
	Yakutat — American vessels			
1972	74.7	2.1	69.9	2.2
1973	68.1	2.4	61.5	2.1
1974	65.0	2.4	60.1	2.2
1975	66.4	1.7	61.9	1.6
1976	52.6	1.6	48.1	1.4
1977	48.8	1.7	44.1	1.6
1978	75.9	2.8	68.9	2.7
1979	97.5	2.9	90.6	2.7
1980	94.6	3.0	82.8	2.5
1981	121.3	4.3	109.8	3.7
1982	105.1	5.0	97.2	4.6
1983	165.4	9.0	152.5	8.3
	Kodiak — American vessels			
1972	80.0	1.4	72.5	1.3
1973	55.4	0.9	50.6	0.8
1974	61.4	1.2	56.9	1.1
1975	59.5	1.1	55.2	1.0
1976	58.0	1.1	53.7	1.0
1977	62.5	1.1	57.7	1.0
1978	73.7	1.1	68.5	1.1
1979	76.0	1.4	69.8	1.3
1980	141.7	3.8	132.3	3.4
1981	158.3	6.0	144.6	4.4
1982	192.6	4.9	180.7	4.7
1983	234.6	7.6	218.0	7.3

$r = 0.985, P < 0.001$), suggesting that both contribute the same amount of information.

Discussion

The empirical distribution of Pacific halibut CPUE is conveniently modeled by a root normal distribution. Because the root normal distribution is positively skewed, the median of the distribution ($m(Y) = \mu^2$) is a better measure of central tendency than the mean ($E(Y) = \mu^2 + \sigma^2$). The estimator of the median derived in this paper assumes that the variance of the square root of catch is proportional to the square root of effort, which appears reasonable in application.

Estimates of the median were approximately 7% less than the estimated mean for regional data between 1972 and 1983. The estimated median was highly correlated with the mean, suggesting that either one could be used. The high correlation results from large sample sizes for most regions and the presence of μ^2 in both the median and the mean of the distribution. The high correlation indicates that σ^2 is constant among years, suggesting consistency in the aggregative tendency of the catching process.

This study indicates that the median estimator is superior to the mean for a skewed distribution. Although both estimators have the same trend, the median provides a safeguard against strongly skewed distributions, has lower sampling variability, and should be considered the estimator of choice, especially for small sample sizes. However, the estimators appear to be somewhat robust to the choice of the underlying distribution, at least among alternatives ranging from lognormality to normality.

In matters of statistical inference involving CPUE data, the underlying root normal distribution invalidates normal statistical inference. CPUE data should be transformed by the square root, and then normal statistical inference can be undertaken. However, if large sample sizes are available, then mean CPUE is approximately normal.

The statistical modeling of CPUE is the first study that should be undertaken to construct an estimator of central tendency of an abundance index. In comparison with studies of other fisheries mentioned in the introduction, two families of underlying statistical distributions emerge.

The first family is characterized as single-modal and positively skewed. For this family, one of the power transformation distributions should sufficiently represent empirical data. For Pacific halibut, the specific distribution is the root normal distribution. Such distributions could arise from modest, not intense, levels of aggregation in fish or fishermen, or from well-developed fisheries where much is known about fish distribution. Also, the root normal distribution has been suggested in conjunction with a Poisson distribution for the catching process and a fixed average fish weight (W. J. Reed, University of Victoria, Victoria, B.C.). In this case, randomness in the catching process also leads to a positively skewed distribution for CPUE.

The second family of distributions is characterized as multimodal with a pronounced mode at the value zero and strongly positively skewed for the rest of the distribution. For this family, a mixture distribution is required along the lines of Pennington (1983), using a probability parameter for the zero value and perhaps one of the power transformation distributions for the other values. Bannerot and Austin (1983) suggested that some function of the probability parameter is most likely related to population abundance. Such distributions would arise from intense levels of aggregation in fish or fishermen or from newly developing fisheries where little is known about the population.

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Appendix

Consider a random variable Y such that $X = Y^{1/2}$ has a normal distribution with mean μ and variance σ^2, denoted $N(\mu, \sigma^2)$. The probability density function of Y is (Parzen 1960, p. 309)

(A1) $$f_Y(y) = [f_X(y^{1/2}) + f_X(-y^{1/2})]/2y^{1/2}, \quad 0 \leq y < \infty$$

where

$$f_X(y^{1/2}) = \exp[-(y^{1/2} - \mu)^2/2\sigma^2]/(2\pi)^{1/2}\sigma.$$

This "root normal" distribution is related to the noncentral χ^2 (derived as the square of $N(\mu, 1)$) with an additional scale parameter. Using the characteristic function of the noncentral χ^2 (Rao 1973, p. 182), the characteristic function of the root normal distribution is

$$\phi(t) = (1 - 2it\sigma^2)^{-1/2} \exp[it\mu^2/(1 - 2it\sigma^2)],$$

from whence come the mean and variance

$$E(Y) = \left.\frac{\partial \ln \phi(t)}{i\partial t}\right|_{t=0} = \mu^2 + \sigma^2$$

$$\text{Var}(Y) = \left.\frac{\partial^2 \ln \phi(t)}{i^2 \partial t^2}\right|_{t=0} = 4\mu^2\sigma^2 + 2\sigma^4.$$

The median $m(Y)$ can be found from the probability statement

(A2) $$P(0 \leq Y \leq m) = 0.5$$

or

$$P(-m^{1/2} \leq Y^{1/2} \leq m^{1/2}) = 0.5$$

TABLE A.1. Comparison of μ^2 with the true median from equation (A2) for various values of μ and σ.

	True median				
σ	$\mu = 8$ $\mu^2 = 64$	10 100	12 144	14 196	16 256
1	64.0	100.0	144.0	196.0	256.0
2	64.0	100.0	144.0	196.0	256.0
4	64.0	100.0	144.0	196.0	256.0
6	64.9	100.1	144.0	196.0	256.0
8	70.6	102.4	144.6	196.1	256.0
10	82.7	110.4	148.7	197.8	256.5
12	100.3	124.8	158.9	203.8	259.6
14	122.4	144.8	175.6	216.3	267.8
16	148.7	169.7	198.1	235.3	282.5

or

$$P[(-m^{1/2} - \mu)/\sigma \leq Z \leq (m^{1/2} - \mu)/\sigma] = 0.5$$

where $Z = (X - \mu)/\sigma$ is $N(0, 1)$. The explicit solution of this equation depends on the values μ and σ. However, if it is assumed that μ is much greater than σ, then the left-hand side of the inequality is insignificant and an approximate solution is

(A3) $$m(Y) = \mu^2.$$

Using values typical of Pacific halibut data, the approximation (A3) is within 10% of the exact solution (A2) when $\mu \geq \sigma$ (Table A.1). Thus, when the transformed data lie at least one standard deviation away from 0, then the median of the root normal distribution can be approximated by the square of the mean of the transformed distribution. Analogously, the median of the lognormal distribution is the exponential of the transformed mean, except that there need be no restriction on the values of μ and σ.

The mode of the distribution, $\max_y f_Y(y)$, is an intractible transcendental function of y. If it is assumed that the negative portion of the transformed distribution $f_X(-y^{1/2})$ in (A1) is insignificant, then the mode is

$$\max_y f_X(y^{1/2})/2y^{1/2}.$$

Differentiating with respect to y and setting equal to 0 yields

$$y - \mu y^{1/2} + \sigma^2 = 0,$$

a quadratic equation of $y^{1/2}$, which has the double solution

$$y^{1/2} = [\mu \pm (\mu^2 - 4\sigma^2)^{1/2}]/2.$$

Only the (+) solution makes sense in light of the assumption.

Bioaccumulation and Bioconversion of Dietary Labeled Fatty Acids in *Artemia* and Winter Flounder (*Pseudopleuronectes americanus*)[1]

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Schauer, P. S., and K. L. Simpson. 1985. Bioaccumulation and bioconversion of dietary labeled fatty acids in *Artemia* and winter flounder (*Pseudopleuronectes americanus*). Can. J. Fish. Aquat. Sci. 42: 1430–1438.

One-day-old *Artemia* were fed for 14 d on nine different rice bran diets, most containing either [1-^{14}C]18:2 ω6 or [1-^{14}C]18:3 ω3, along with various nonlabeled fatty acids. The 15-d-old *Artemia* were then fed as labeled diets to winter flounder (*Pseudopleuronectes americanus*). Although 18:2 ω6, 18:3 ω3, and 20:5 ω3 were primarily bioaccumulated, all 15-d-old *Artemia* groups contained some 22:6 ω3, even when it was not present in the diet. Therefore, the 15-d-old *Artemia* had bioconverted small amounts of 18:2 ω6 and 18:3 ω3 to 20:5 ω3 and 22:6 ω3. The bioconversion rate was substantially reduced when either 20:5 ω3 or 22:6 ω3 was present in the diet. When fed the 15-d-old *Artemia*, the winter flounder accumulated 20:5 ω3 and 22:6 ω3 and also bioconverted some of the 18:3 ω3 to 20:5 ω3 and 22:6 ω3. Those fish fed diets that contained lower levels of 20:5 ω3 and 22:6 ω3 contained higher levels of radioactively labeled 20:5 ω3 and 22:6 ω3. When 20:5 ω3 and 22:6 ω3 were present from the diet, the radioactivity generally accumulated in the 20:4 ω3 and 20:5 ω3 fatty acids.

Neuf diètes au son de riz contenant pour la plupart du [1-^{14}C]18:2 ω6 ou du [1-^{14}C]18:3 ω3 ainsi que d'autres acides gras non marqués ont été administrées pendant 14 jours à des *Artemia* âgées d'un jour. Les *Artemia* de 15 jours ont ensuite servi, comme aliment marqué, à alimenter des plies rouges (*Pseudopleuronectes americanus*). Les 18:2 ω6, 18:3 ω3 et 20:5 ω3 étaient surtout accumulés, mais tous les groupes d'*Artemia* de 15 jours contenaient une certaine quantité de 22:6 ω3, même si cet acide était absent de leur diète. Ces *Artemia* avaient donc converti de petites quantités de 18:2 ω6 et de 18:3 ω3 en 20:5 ω3 et en 22:6 ω3. Le taux de cette bioconversion était réduit de façon appréciable en présence de 20:5 ω3 ou de 22:6 ω3 dans la diète. Les plies rouges dont la diète était constituée d'*Artemia* de 15 jours ont accumulé du 20:5 ω3 et du 22:6 ω3 et ont converti une partie du 18:3 ω3 en 20:5 ω3 et en 22:6 ω3. Les poissons dont la diète contenait des teneurs plus faibles en 20:5 ω3 et en 22:6 ω3 présentaient des teneurs plus élevées en 20:5 ω3 et en 22:6 ω3 marqués. Lorsque la diète contenait du 20:5 ω3 et du 22:6 ω3, la radioactivité se retrouvait généralement dans les acides gras 20:4 ω3 et 20:5 ω3.

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Artemia are extensively used as a food source for most laboratory-cultured larval and juvenile marine fish and crustaceans. Nauplii are generally used as the only food for larval fish stages, while cultured or field-collected adults are often fed to juveniles or older animals. However, not all *Artemia* strains are nutritionally equal or promote the same survival and growth in various marine organisms (Beck et al. 1980; Beck and Bengtson 1982; Klein-MacPhee et al. 1980; Johns et al. 1978, 1980, 1981a, 1981b). Differences in the efficacy of a particular geographic strain of *Artemia* may be due to the size of the newly hatched nauplii (Vanhaecke and Sorgeloos 1980; Beck and Bengtson 1982), the occurrence of various contaminants which may vary with the different collection sites (Bookhout and Costlow 1970; Olney et al. 1980), the nutritional value of the nauplii, particularly with reference to their fatty acid content (Watanabe et al. 1978, 1980; Fujita et al. 1980), or a combination of the latter two features (Schauer et al. 1980).

Watanabe et al. (1978) suggested that *Artemia* nauplii that contain 18:3 ω3 as their predominant long-chain (LC) polyunsaturated fatty acid (PUFA) were good only for freshwater consumer organisms, while those that contain mostly 20:5 ω3 were good for marine fish and crustaceans, as well as freshwater organisms. This would appear to be logical, because marine fish generally require a dietary source of 20:5 ω3 for proper growth (Owen et al. 1972, 1975; Yone 1976; Scott and Middleton 1979; Fujita et al. 1980; Watanabe et al. 1980). Therefore, culturists of marine fish must ensure that their nauplii or field-collected/cultured *Artemia* contain adequate levels of 20:5 ω3.

Generally, the fatty acid composition of cultured *Artemia* reflects the composition of their diet (Claus et al. 1979; Watanabe et al. 1978, 1980; Fujita et al. 1980), and most of the

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TABLE 1. Percent composition of the rice bran diets.

Components	Diet								
	0	1	2	3	4	5	6	7	8
Rice bran solids	95.0	92.8	92.8	92.8	92.8	92.8	92.8	92.8	92.8
Rice bran oil	3.0	5.2	4.2	4.2	4.2	3.7	3.7	3.7	3.2
FAME									
10:0	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
12:0	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
18:2 ω6[a]	—	—	1.0	—	—	1.0	—	—	—
18:3 ω3[a]	—	—	—	1.0	—	—	1.0	1.0	1.0
20:5 ω3	—	—	—	—	—	—	0.5	—	0.5
22:6 ω3	—	—	—	—	1.0	0.5	—	0.5	0.5

[a][1-^{14}C]18:2 ω6 and [1-^{14}C]18:3 ω3 were added to the 1.0% cold FAME at approximately 2.2×10^7 Bq/g diet for 18:2 ω6 and 0.82×10^7 Bq/g diet for 18:3 ω3.

diets commonly used in laboratory culture of *Artemia* contain little or no 20:5 ω3 (Dobbeleir et al. 1980; Sorgeloos et al. 1980). Furthermore, it has not been shown conclusively that *Artemia* can bioconvert dietary 18:3 ω3 to the levels of 20:5 ω3 needed to provide adequate nutrition for a consumer organism. Reports that *Artemia* are able to convert 18:3 ω3 to 20:5 ω3 and 22:6 ω3 are not well founded because researchers drew their conclusions from fatty acid profiles, often without regard to the initial naupliar content (Hinchcliffe and Riley 1972; Kayama et al. 1963b; Jezyk and Penicnak 1966), the absolute amounts of particular fatty acids (Watanabe et al. 1978), or the effect of the diet on the *Artemia* fatty acid profile (Dobbeleir et al. 1980).

This study was designed to characterize some aspects of the fatty acid metabolism by *Artemia* and winter flounder (*Pseudopleuronectes americanus*). This research was undertaken to determine both whether *Artemia* predominantly bioaccumulate their dietary fatty acids or whether they have the ability to bioconvert 18:2 ω6 and 18:3 ω3 to 20:5 ω3 and 22:6 ω3 and whether winter flounder bioaccumulate their dietary fatty acids or bioconvert them to satisfy their needs for LC PUFAs.

Materials and Methods

Rice Bran Diet Preparation

Powdered rice bran was used as the diet for *Artemia* because it is one of the more practical and inexpensive diets (Dobbeleir et al. 1980; Sorgeloos et al. 1980). Rice bran, which contained about 17% lipid, was extracted with petroleum ether (PE) by a Soxhlet refluxing procedure for 16 h to remove all lipids and pesticide residues. The PE–lipid solution was then extracted with acetonitrile to remove pesticides (Food and Drug Administration 1975). The rice bran oil, as a PE solution, was then added back to the rice bran solids at 3.0% (diet 0) and 5.2% (diet 1) along with the fatty acid methyl esters (FAMEs) 10:0 and 12:0 (Table 1). These diets constituted the control diets. The treatment diets consisted of the addition of 18:2 ω6, 18:3 ω3, 20:5 ω3, and/or 22:6 ω3 to the control diet at 0.5 or 1.0% of the diet, along with a radioactive source of either [1-^{14}C]18:2 ω6 or [1-^{14}C]18:3 ω3 (New England Nuclear, Boston, MA), 2.2×10^7 at 0.60 Bq/g diet or 0.82×10^7 Bq/g diet, respectively. The radioactive fatty acids were dissolved in benzene and then added to appropriate aliquots to give the desired level of radioactivity. The solvents were then flash evaporated and the diet was desiccated for at least 24 h before use.

The fatty acid profiles of each diet are presented in Table 2. A check of the purity of the radioactivity source and the accuracy of the separation techniques using silver nitrate thin-layer chromatography (TLC) showed that better than 97.6% of the spiked source was in the proper TLC band (Table 3). Generally, less than 1.4% of the radioactivity source was in the higher numbered bond bands (4, 5, and 6), indicating good TLC band separations and a reasonably high purity of the labeled fatty acids.

Artemia Culture Systems

Artemia cysts (Shark Bay, Australia, World Ocean Ltd., harvested 1978, lot No. 113) were incubated for 29 h (Johns et al. 1980) in a 250-mL separatory funnel filled with filtered seawater (30‰, 25 ± 1°C). One-day-old nauplii were stocked at 1 nauplius/mL (determined by aliquot counts from a large volume of nauplii) in twenty 1-L Erlenmeyer flasks supplied with continuous aeration.

The *Artemia* in each flask were fed once per day at a level of 0.1 g diet·d^{-1}·1000 nauplii^{-1} (Johnson 1980). One half of the water was exchanged with new seawater every third day. Water exchanges were made up to the point of the radioactive diet feedings, after which time no water was changed until the end of the feeding study. Twice daily, the diet that had settled out of solution was manually swirled back into suspension.

One-day-old *Artemia* were fed diet 0 for 7 d to reduce the level of 20:5 ω3 and then one of the diet treatments for an additional 7 d (15-d-old *Artemia*). The beginning nauplii and the 15-d-old *Artemia* were analyzed for their fatty acid and radioactivity content.

Winter Flounder Culture and Feeding

Field-collected winter flounder juveniles (2.91 ± 0.71 g; 63.6 ± 4.71 mm FL) were taken by beach seine from the lower basin area of Narrow River in July 1980 and analyzed to provide a reference fatty acid profile to compare with cultured fish.

Seventy-five-day-old (postmetamorphosed) winter flounder juveniles (average length 13.3 ± 4.4 mm) used in this study had been cultured from laboratory-spawned eggs of field-collected adults according to the procedures described by Klein-MacPhee et al. (1980). They were fed 1-d-old Australian *Artemia* for 2 wk prior to the onset of this experiment and then were stocked in eight 10-L black plastic tubs at a density of 12 fish per tub. Fine

TABLE 2. Fatty acid composition (% weight) of rice bran oil and the rice bran diets.

FAME	Rice bran oil	Diet 0	1	2	3	4	5	6	7	8
10:0	—	9.42	6.64	5.50	5.59	7.22	5.54	4.64	4.17	3.79
12:0	0.03	15.94	20.69	18.10	20.39	26.95	21.54	17.70	18.25	17.31
14:0	0.52	0.13	0.20	0.22	0.12	0.17	0.12	0.28	0.16	0.11
14:1	0.02	0.01	—	0.17	0.14	—	0.23	0.21	0.17	0.22
16:0	17.25	13.23	12.65	9.41	7.53	9.06	6.61	8.79	8.55	6.27
18:0	1.48	1.22	0.91	1.19	0.78	0.87	0.75	0.98	0.88	0.59
18:1 ω9	40.18	31.59	35.64	29.18	27.30	25.94	21.88	25.62	24.42	22.54
18:2 ω6	35.18	25.29	22.10	35.11	20.78	17.92	33.80	18.51	17.98	16.48
18:3 ω3	2.60[a]	1.30[b]	0.64[c]	0.52[d]	17.00	0.60	0.10	15.36	16.96	16.12
20:5 ω3	—	—	—	—	—	—	—	7.91	—	9.07
22:6 ω3	—	—	—	—	—	10.86	9.38	—	8.42	7.48
FAME (mg/g)	73.74	41.95	52.16	48.96	52.82	56.24	40.17	51.43	45.38	49.42
% lipid	16.02	7.19	7.79	7.83	7.54	7.46	7.28	7.86	7.65	7.64

[a]Plus 1.07% 18:3 ω6, 0.30% 20:2 ω6/20:3 ω6, 0.75% 22:1 and 0.64% unknown.
[b]Plus 0.58% 18:3 ω6, 0.18% 20:2 ω6/20:3 ω6, 0.06% 22:1 and 0.73% unknown.
[c]Plus 0.52% 20:1.
[d]Plus 0.58% 20:1.

TABLE 3. Radioactivity (kBq) of the rice bran diets.

	Diet 2	3	5	6	7	8
No. of double bonds						
0 + 1	0.10	0.02	0.14	0.02	0.02	0.01
2	11.85	0.00	22.33	0.15	0.13	0.08
3	0.05	7.77	0.10	8.68	9.98	8.77
4, 5, 6	0.00	0.10	0.00	0.00	0.10	0.12
kBq recovered/g	12.00	7.89	22.57	8.85	10.23	8.98
% recovery	51.4	78.2	82.5	91.5	89.8	76.7
Total kBq/g	23.3	10.09	27.36	9.67	11.39	11.71

mesh screens (400 μm) were used to cover the tops of the tubs to keep the fish from jumping out. Each tub contained 6 L of filtered (0.25 μm), ultraviolet-treated seawater (30‰). Three litres of the static seawater was replaced every third day by siphoning, which also removed feces and any unconsumed food.

The 75-d-old flounder were fed 8- to 14-d-old *Artemia* that had been raised on a low-lipid (3% total lipid) rice bran diet that contained no PUFAs other than those common to the rice bran oil for 39 d before being used in this diet study. Prior to the initiation of the feeding of the labeled *Artemia* diets, the density of fish was reduced to 8 fish per tub and the temperature was raised, in increments, from 5.5 + 0.5 to 14.0 + 0.5°C to resemble the ambient water temperature changes in Narragansett Bay, RI. The flounder were starved for 3 d prior to being fed the labeled *Artemia*.

The 15-d-old *Artemia* were fed to the flounder for 3 d at approximately 330 *Artemia* per day. One day after the last feeding, four fish that had been fed the 15-d-old *Artemia* were removed from each tub, weighed, measured, and stored at −20°C for analysis. Only the winter flounder that were fed *Artemia* diet 3 were analyzed for this sampling time. They were designated "flounder fed *Artemia* diet 3a." One day is considered enough time for the flounder to void its stomach of remaining food (Kiorbe 1978). The other four fish remained in the static culture without additional feeding or water changes for another 6 d and were then collected and processed in a similar manner. The flounder that had been fed *Artemia* diet 3 and starved for 6 d were referred to as "flounder fed *Artemia* diet 3b," while all the other flounder groups collected at this time were referred to by only a diet number, without a letter designation.

Analytical Procedures

All analyses for the total lipids, FAME profiles, and radioactivity of the nauplii and 15-d-old *Artemia* were performed in triplicate, and the data were presented as means of three sample analyses. Approximately 1 g of rice bran or 0.5 g of *Artemia* (wet weight) was extracted twice with chloroform–methanol–water (1:2:0.8, by volume, adjusted for moisture in the sample) according to the Bligh and Dyer technique, as modified by Kates (1972). The preparation of the FAMEs was as described by Schauer and Simpson (1979). The analyses of the FAMEs was made by gas–liquid chromatography. Identifications were initially made on a GP 10% SF 2330 column and later confirmed on an ethylene glycol succinate polyester column.

The FAMEs were fractionated according to their degree of unsaturation via silver nitrate TLC modeled after the procedures

of Dudley and Anderson (1975). All silver nitrate (14%) impregnated silica gel plates (Supelco, Inc., Bellefonte, PA) were preconditioned by heating at 105°C for more than 1 h and stored in a plate desiccator, if not used immediately. Samples were spotted on the plates with 50-μL capillary tubes. The plates were run one full length of the plate in a PE – ethyl ether – acetic acid solvent system (78:22:1), followed by three successive shorter runs (2.0 cm up the plate) in an ethyl ether – acetic acid solvent system (90:10). The TLC chromatograms contained seven distinct horizontal bands, each band corresponding to a group of FAMEs with a similar degree of unsaturation. The most unsaturated FAMEs, those with six double bonds (22:6 ω3), occurred in the band at the bottom of the plate. The next band (up the plate) contained five double bond type FAMEs (20:5 ω3 and 20:5 ω3) and so on. The top band, at the solvent front, contained all the saturated FAMEs. The second TLC solvent system was used to increase the separation of the five and six double bond bands which were nearest the spotting point. These two LC PUFA groups were not always totally separated, and therefore, the counts attributed to 22:6 ω3 may occasionally include a small amount of the 20:5 ω3 and/or 22:5 ω3 counts. Nitrogen was used to dry the plates between runs to prevent autoxidation of the PUFAs.

Visualization of the bands on the TLC plates was made by spraying the plates with bromothymol blue and exposing them to ammonia vapor. Standard FAME mixtures were used for positive identification on every plate. Each double bond band was scraped into a culture tube containing 10 mL of 2.0% acetic acid – methanol, flushed with nitrogen, and thoroughly mixed on a vortex mixer. The solution was then heated for 5 min in a boiling water bath. After cooling, 20 mL of water and 10 mL of acetone were added to each tube and the FAMEs were extracted from the aqueous phase with 5 mL of PE (three times).

The total volume of PE was evaporated and the lipid material was made up to a known volume for injection in a gas–liquid chromatograph to confirm band identification and FAME purity. After the injection solution was again evaporated to near dryness, 10 mL of Aquasol II (New England Nuclear, Boston, MA) was added, and the sample was counted for 20 min in a Packard TriCarb scintillation counter (model 3255) on the 14C/14Cq mode. The counts per minute (CPM) were then matched with the FAME content of that band to correlate the amount of radioactivity associated with a particular FAME group. The counting efficiency was usually greater than 75%. The CPM values associated with each double bond band were corrected for sample counting efficiency, using a standard curve, and then converted to disintegrations per minute (Bq × 60) and adjusted to a gram weight basis (wet weights). The Bqs were later adjusted for the TLC recovery efficiency from the silver nitrate TLC process (known counts applied to the plate less counts recovered/100) to get the total Bq × 60 [1-^{14}C] label per gram of sample. Recovery from the TLC procedure averaged 78.6% of the applied counts.

Results

Freshly hatched Australian *Artemia* nauplii contained 12.0% 18:3 ω3 and 7.8% 20:5 ω3, but no 22:6 ω3 (Table 4). They also contained higher levels of total lipids (3.2%) and FAMEs (22.0 mg/g) than any of the 15-d-old *Artemia* fed the rice bran diets. The 15-d-old *Artemia* contained on the average 2.1% and 5.7 mg/g total lipids and FAMEs, respectively.

Although all the diets were rich in 10:0 and 12:0 (Table 1), these FAMEs did not accumulate in the 15-d-old *Artemia* and were probably used for energy. The levels of 18:2 ω6 in the *Artemia* fed diets 2 and 5 supplemented with 18:2 ω6 were two times higher than those in the *Artemia* fed diets 3, 6, 7, and 8 supplemented with 18:3 ω3. Interestingly, *Artemia* fed diet 1, which contained only the natural level of 18:2 ω6 found in the rice bran oil, also contained a relatively high level of 18:2 ω6. Apparently, the lack of other LC PUFAs induced the accumulation of 18:2 ω6.

Artemia that were fed diets 3, 6, 7, and 8, all supplemented with 18:3 ω3, contained levels of that fatty acid that were nearly 10 times greater than levels in *Artemia* fed diets 1, 2, 4, and 5, without 18:3 ω3 supplementation. The group fed on diet 3 contained a slightly lower 18:3 ω3 level than the other groups that received diets supplemented with 18:3 ω3, and other LC PUFAs, probably as a result of a bioconversion of 18:3 ω3 to accommodate for the lack of the LC PUFAs. The fatty acid data indicate that some of the 18:2 ω6, 18:3 ω3, and/or 18:4 ω3 was bioconverted to LC PUFAs.

The levels of 20:5 ω3 in the 15-d-old *Artemia* fed diets 6 and 8 were proportional to the diet levels, as was the accumulation of the 18:2 ω6 and 18:3 ω3. Accumulation of 22:6 ω3, however, was not proportional to the amount in the diets (4, 5, and 7). The supplementation of 22:6 ω3 resulted in *Artemia* that contained higher levels of 20:5 ω3 than groups not fed diets containing 20:5 ω3 and 22:6 ω3. However, all 15-d-old *Artemia* groups contained some 20:5 ω3 and 22:6 ω3 even when it was not present in the diets.

The fortification of the rice bran diet with 18:3 ω3 and 20:5 ω3 (diet 6) or 18:3 ω3 and 22:6 ω3 (diet 7) reduced total lipid accumulation in the *Artemia*. The combination of 18:3 ω3, 20:5 ω3, and 22:6 ω3 in diet 8 produced a high level of FAME and total lipids in the 15-d-old *Artemia*, most likely because there was an excess of the LC PUFAs in their diet. It appears that 20:5 ω3 is preferentially accumulated, but that 22:6 ω3 is important, but at relatively low levels.

A major portion of the radioactivity remained as [1-^{14}C]18:2 ω6 or [1-^{14}C]18:3 ω3 (68–83%) or was metabolized (8.1–15.4%) and appeared in shorter chained fatty acids (Table 5). Less than 20% of the label was ever found in the LC PUFAs. Therefore, the radioactivity data substantiated the primary accumulation of 18:2 ω6 and 18:3 ω3 in the 15-d-old *Artemia*, as indicated by the fatty acid data; however, these data also indicate that the 15-d-old *Artemia* did bioconvert some of both 18:2 ω6 and 18:3 ω3 to 20:5 ω3 and 22:6 ω3. The greatest percentage of [1-^{14}C] label movement was found in *Artemia* fed diet 3. The *Artemia* probably compensated for the lack of 20:5 ω3 and 22:6 ω3 (diet 3) by bioconverting 18:3 ω3 to these LC PUFAs. The addition of 20:5 ω3 in diet 6, 22:6 ω3 in diet 7, and the presence of 20:5 ω3 and 22:6 ω3 in diet 8 reduced the total amount of the label found in 20:5 ω3 and 22:6 ω3. Therefore, the presence of LC PUFA in the diets obviates the bioconversion mechanism. For diets that contained 20:5 ω3 or 22:6 ω3, the radioactivity accumulated in the fifth double bond band as 20:5 ω3 and not as 22:5 ω3.

The level of total radioactivity (kBq × 60) in the *Artemia* fed diets 2 and 5 which contained [1-^{14}C]18:2 ω6 was higher than the level of radioactivity in the *Artemia* fed diets 3, 6, 7, and 8 which contained [1-^{14}C]18:3 ω3 because the specific activities of the 18:2 ω6 label was higher than that of the 18:3 ω3 label. Although the [1-^{14}C]18:2 ω6 was bioconverted to longer chain unsaturated PUFAs, the radioactivity was associated with only one ω6 FAME (20:4 ω6), while the other radioactive LC PUFAs

TABLE 4. Fatty acid composition (% weight) of newly hatched *Artemia* and 15-d-old *Artemia*.

FAME	Newly hatched *Artemia*	*Artemia* fed diet							
		1	2	3	4	5	6	7	8
10:0	—	1.96	1.06	Trace	0.20	1.37	Trace	1.59	2.26
12:0	0.25	1.66	0.74	0.67	0.78	0.56	0.73	1.34	2.14
14:0	1.14	1.54	1.24	1.78	1.49	2.59	1.27	2.53	4.17
14:1	1.76	1.30	—	1.56	0.96	—	0.35	—	0.02
16:0	13.12	9.86	11.02	12.06	9.82	9.16	9.08	8.82	12.51
16:1	12.64	6.42	3.60	6.09	6.03	6.69	4.64	5.73	5.35
16:2 ω4/16:3 ω7	3.88	0.71	0.17	0.05	0.19	0.14	0.14	0.27	0.08
18:0	3.89	4.60	7.36	6.34	5.35	5.50	5.88	6.48	6.45
18:1 ω9	31.86	41.37	39.06	39.13	40.30	35.33	38.32	35.35	30.88
18:2 ω6	8.02	22.27	27.76	12.70	17.21	25.43	12.93	12.59	10.44
18:3 ω3	12.02	2.22	0.95	11.09	1.25	1.66	12.41	13.71	12.95
18:4 ω3	4.06	—	—	—	—	—	—	—	—
20:3 ω3/20:4 ω6[a]	—	2.14	3.86	3.38	0.94	2.26	1.69	2.50	1.36
20:5 ω3	7.80	3.27	2.89	1.75	9.23	7.49	11.84	5.97	9.56
22:6 ω3	—	0.68	0.29	3.32[b]	5.73[c]	1.82	0.72	3.03	1.83
FAME (mg/g)	22.04	7.06	4.42	6.04	5.46	5.55	5.84	4.95	6.32
% lipid	3.19	2.14	1.69	2.85	2.53	1.61	1.35	1.90	2.72

[a]Trace amounts of 20:4 ω3 were found in all 15-d-old *Artemia* groups.
[b]Plus 0.08% 20:2 ω6/20:3 ω6.
[c]Plus 0.40% 20:1 and 0.12% 22:1.

TABLE 5. Radioactivity (kBq) in 15-d-old *Artemia*.

	Artemia fed diet					
	2	3	5	6	7	8
No. of double bonds						
0 + 1	0.19	0.06	0.51	0.07	0.10	0.08
2	1.93	0.02	2.37	0.01	0.02	0.04
3	0.03	0.43	0.12	0.64	0.92	0.61
4	0.07	0.02	0.07	0.01	0.01	0.02
5	0.05	0.04	0.11	0.06	0.07	0.03
6	0.05	0.07	0.14	0.03	0.02	0.02
kBq recovered/g	2.25	0.64	3.32	0.82	1.14	0.80
% recovery	76.1	70.7	65.5	80.7	60.1	57.9
Total kBq/g	2.96	0.91	5.07	1.02	1.90	1.38

were identified as ω3 FAMEs (20:5 ω3 and 22:6 ω3). The *Artemia* fed diet 5, which contained supplemented levels of 22:6 ω3 along with [1-^{14}C]18:2 ω6, metabolized a greater amount of the labeled compounds than did *Artemia* fed diet 2 (without 22:6 ω3 supplementation).

The amount of catabolism of the labeled compounds was directly related to the presence or absence of the LC PUFAs. For instance, in the diet 2 group the level of breakdown associated with the labeled fatty acid was lower compared with the diet 5 group when 22:6 ω3 was present. Similarly, the amount of catabolism increased slightly in a stepwise fashion in diets 6, 7, and 8 which contained increasing levels of the LC PUFAs.

Winter Flounder Fed *Artemia* Diets

Juvenile, field-collected winter flounder contained 16.3% 20:5 ω3, 5.92% 22:5 ω3, and 11.9% 22:6 ω3 (Table 6). The total lipid level was 1.68%. Eggs that were spawned naturally from gravid field-collected adults also contained high levels of the ω3 PUFAs, but the percentage of each ω3 fatty acid was lower than in the field-collected fish. The level of 16:0 in the eggs was nearly twice that of the field-collected fish, indicating the importance of this fatty acid to the developing larvae, probably as energy.

In the 74-d-old flounder, which had been fed 1-d-old Australian *Artemia* for 2 wk prior to their collection for analysis or use in the feeding study, the FAME profile resembled that of their diet more than their original egg profile (Table 6). For example, the levels of 18:1 ω9, 18:2 ω6, and 18:3 ω3 were all higher, while the levels of 16:0, 18:0, 20:3 ω3/20:4 ω6, 22:5 ω3, and 22:6 ω3 were lower. The lack of 22:5 ω3 and 22:6 ω3 in the *Artemia* nauplii apparently resulted in the near absence of these fatty acids in the 75-d-old flounder. The amount of FAME was about 3.8 times higher and the total lipid was about 2.6 times higher in the 75-d-old flounder fed the 1-d-old *Artemia* than the levels found in the wild flounder.

The total FAME level in the flounder fed the 8- to 14-d-old *Artemia* for 39 d and then 15-d-old labeled *Artemia* for 3 d (127-d-old flounder) and subsequently starved for 6 d was

TABLE 6. Fatty acid composition (% weight) of winter flounder fed 15-d-old *Artemia* (NA = data not available).

FAME	Wild fish	0-d-old fish	75-d-old fish	Winter flounder fed *Artemia* diet							
				1	2	3	4	5	6	7	8
14:0	2.33	2.74	1.57	1.68	2.11	1.93	5.34	6.41	2.72	3.05	4.28
14:1	2.32	1.92	1.24	—	0.90	0.77	—	2.47	0.68	1.20	2.94
16:0	15.40	31.87	17.34	16.58	14.76	13.60	18.15	15.05	16.92	15.14	16.85
16:1 ω7	9.87	9.00	11.71	5.70	6.71	4.41	7.60	6.72	5.90	5.79	5.40
16:2 ω4	1.96	1.11	1.80	—	—	—	—	—	—	—	—
18:0	6.31	7.78	4.09	10.59	6.58	11.11	10.58	10.14	12.90	9.53	9.75
18:1 ω9	12.92	15.11	38.40	27.90	26.45	26.25	31.19	16.08	26.51	21.02	18.52
18:2 ω6	2.07	1.79	5.98	8.13	7.41	10.16	7.62	8.06	7.54	6.67	6.73
18:3 ω6	—	—	1.59	—	—	0.49	1.81	3.85	2.11	1.21	1.86
18:3 ω3	—	—	9.10	0.87	0.70	3.15	—	—	0.51	0.70	0.10
20:1	4.50	1.26	—	3.78	2.80	0.52	2.91	3.91	0.29	0.82	1.42
20:3 ω6/20:4 ω6	4.59	3.67	1.32	8.95	8.43	9.05	3.79	13.30	10.01	10.27	9.08
22:1[a]	—	—	—	1.58	3.70	2.04	0.67	—	—	—	—
20:5 ω3	16.27	10.84	5.61	7.97	11.30	11.35	3.64	8.92	11.06	18.56	18.41
22:5 ω3	5.92	3.38	0.25	2.47	2.44	2.25	0.06	0.29	0.51	2.16	1.61
22:6 ω3	11.90[b]	9.53	Trace	3.77	5.70	2.92	5.83[c]	1.96[d]	1.34	3.88	3.02
FAME (mg/g)	4.55	4.94	17.3	5.18	5.55	5.43	9.90	8.20	3.41	3.02	5.45
% lipid	1.68	NA	4.31	1.83	1.41	1.86	1.88	1.82	1.52	1.40	1.31

[a]Trace amounts of 20:4 ω3 were present in all winter flounder groups.
[b]Plus 2.43% 22:4 ω6.
[c]Plus 0.78% 12:0.
[d]Plus 2.55% 20:2 ω6/20:3 ω6 and 0.30% 22:4 ω6.

TABLE 7. Radioactivity (Bq) in winter flounder fed 15-d-old *Artemia*.

	Winter flounder fed *Artemia* diet						
	2	3a	3b	5	6	7	8
No. of double bonds							
0 + 1	4.30	5.97	6.87	15.83	7.97	4.60	3.88
2	14.82	5.50	20.66	48.83	5.85	4.22	4.10
3	4.95	28.83	17.43	10.83	14.68	9.02	7.72
4	3.70	4.98	25.83	10.43	1.43	3.80	1.80
5	2.97	6.65	5.98	12.25	11.20	3.20	3.33
6	6.80	9.07	8.35	6.53	2.97	3.28	2.28
Bq recovered/g	37.54	56.0	85.12	104.7	44.10	28.12	23.11
% recovery	76.9	89.3	80.4	72.3	100	81.5	90.2
Total Bq/g	48.82	62.71	105.87	144.8	421.1	34.51	25.62

generally 2–3 times lower and the total lipid level was 2.5–3.0 times lower than in the 75-d-old flounder (Table 6). Fish fed diets with no 18:3 ω3 but with 22:6 ω3 (diets 4 and 5) accumulated the highest level of FAME and total lipids. The flounder fed diets that contained 18:3 ω3, 20:5 ω3, and/or 22:6 ω3 (diets 6, 7, and 8) had the lowest level of both total lipid and FAMEs.

The levels of 20:5 ω3, 22:5 ω3, and 22:6 ω3 in flounder fed *Artemia* diet 1 increased substantially from the levels in the 75-d-old flounder (Table 6). These higher levels may have been due to a preferential accumulation, in the case of 20:5 ω3 and 22:6 ω3; however, this would not account for the increased level of 22:5 ω3, since it was not present in the *Artemia* diet. When flounder were fed *Artemia* diet 2, which contained slightly more 18:2 ω6 and less 18:3 ω3 than *Artemia* diet 1, they contained lower levels of 18:0 but higher levels of 20:5 ω3 and 22:6 ω3 than flounder fed *Artemia* diet 1. In the fish fed the *Artemia* diets that contained higher 18:3 ω3 levels (diets 3, 6, 7, and 8), 20:5 ω3 was the predominant PUFA.

In flounder fed *Artemia* diets 4 and 5, without substantial levels of 18:3 ω3 (Table 1), 20:5 ω3 was generally lower than in the other flounder groups, even though all the *Artemia* diets contained substantial amounts of 20:5 ω3. The presence of higher levels of 18:3 ω6 and 20:3 ω6/20:4 ω6 in flounder fed *Artemia* diet 5 indicates that dietary 18:2 ω6 was being elongated and desaturated. Further evidence for that finding was the occurrence of 22:4 ω6 in the flounder fed *Artemia* diet 5.

Proportionally more of the radioactivity was found in the four, five, and six double bond bands (20:4 ω3, 20:5 ω3, and 22:6 ω3, respectively) in the flounder (Table 7) than in the *Artemia* diets (Table 5). The label appeared to concentrate predominantly in the 20:5 ω3 and 22:6 ω3 position. Also, a substantial proportion of the existing label occurred in the 0 + 1 and 2 double bond positions, indicating a breakdown of the

labeled 18:3 ω3. In all but the flounder fed *Artemia* diet 5, the percent of label in 18:3 ω3 in the test flounder was less than one-half that of their diets. An additional 6 d of starvation (flounder fed *Artemia* diet 3b compared with flounder fed *Artemia* diet 3a) resulted in greater movement to the LC PUFA positions (sum of radioactivity in the fourth, fifth, and sixth double bond bands) as well as more breakdown (bands 0 + 1 and 2) (Table 7).

The two diets with the lowest levels of 20:5 ω3 and 22:6 ω3, *Artemia* diets 2 and 3, resulted in the greatest movement of the label. The presence of 20:5 ω3 or 22:6 ω3 in the labeled diets generally reduced the movement of radioactivity to the 22:6 ω3 position and resulted in a far greater concentration at the 20:5 ω3 position.

Discussion

Dietary 22:6 ω3 did not accumulate in the 15-d-old *Artemia* in the same manner as 18:2 ω6, 18:3 ω3, or 20:5 ω3. In fact, when *Artemia* were fed 22:6 ω3 as the only LC PUFA, they produced higher levels of 20:5 ω3 than 22:6 ω3. Nevertheless, all 15-d-old *Artemia* contained some 22:6 ω3, even when not present in all diets.

A low level of 22:6 ω3 appears to have a sparing action on the metabolism or bioconversion of 20:5 ω3. Therefore, after an initial level of 22:6 ω3 is reached, 20:5 ω3 accumulates without being modified. Perhaps, 22:6 ω3 serves a particular function in membranes or tissues related to a reproductive activity; however, it is not generally deposited in the eggs, since cysts have seldom been reported to contain 22:6 ω3 (Watanabe et al. 1978, 1980; Schauer et al. 1980). The excess dietary 22:6 ω3 was probably catabolized for energy.

Diets with only 18:3 ω3 or 22:6 ω3 promoted substantial lipid accumulation and resulted in greater bioconversion activity than did the other diets. Those diets with either 18:3 ω3 and 20:5 ω3 (diet 6) or 18:3 ω3 and 22:6 ω3 (diet 7) resulted in the lowest lipid accumulation. If the lower lipid accumulation is a result of a better balanced diet, which meets the lipid nutritional needs of the *Artemia*, then it appears that a diet for cultured *Artemia* should include 1.0% 18:3 ω3 and 0.5% 20:5 ω3, as well as other less-saturated and shorter chain fatty acids.

In the present study, 15-d-old *Artemia* were shown to bioconvert dietary 18:2 ω6 and 18:3 ω3 to 20:5 ω3 and 22:6 ω3. The conversion of 18:3 ω3 to 20:5 ω3 and 22:6 ω3 is common for a number of crustaceans, for example the mysid (*Gnathapausia* sp.) and a decapod (*Acanthepura purpurea*) (Morris and Sargent 1973), two euphausiids (*Nematabrachion sexspinosus* (Morris and Sargent 1973) and *Thysanoessa inermis* (Henderson et al. 1981)), two calanoid copepods (*Calanus helgolandicus* (Lee et al. 1971) and *Paracalanus parvus* (Moreno et al. 1979a)), and the prawn (*Penaeus japonicus*) (Kayama et al. 1980).

The unusual movement of 18:2 ω6 into labeled 20- and 22-carbon ω3 LC PUFAs is possible via a number of pathways. Moreno et al. (1979b) postulated that the diatom *Phaeodactulum tricornutum* can biosynthesize 18:3 ω3 directly from 18:2 ω6 via an ω3 desaturase mediated reaction. They also pointed out that 18:3 ω3 could also have been biosynthesized from 12:3 ω3 by a number of elongation and desaturation reactions. This has been suggested to be the main pathway in spinach, barley, and yeast (Jacobsen et al. 1973a, 1973b; Kannangara et al. 1973). However, this is probably not the prevalent mechanism in aquatic organisms, because neither 12:3 ω3, 14:3 ω3, nor 16:3 ω3 are commonly found as component fatty acids in fish or crustaceans. It is more likely that 18:2 ω6 is converted directly to 18:3 ω3, or that a two-carbon unit which contained the label is cleaved from the [1-^{14}C]18:2 ω6 and reincorporated into an ω3 fatty acid which leads to the [1-^{14}C]-labeled 22:6 ω3.

A number of workers have concluded that *Artemia* can bioconvert 18:3 ω3 to 20:5 ω3 and 22:6 ω3. However, some of these reports contain insufficient data from which to draw that conclusion (Hinchcliffe and Riley 1972; Kayama et al. 1963b; Jezyk and Penicnak 1966), or are based solely on changes in absolute amounts of particularly fatty acids (Watanabe et al. 1978), or do not consider changes in the percent composition as a result of a dietary influence (Dobbeleir et al. 1980). Using radioactive tracers, it has now been shown that *Artemia* can produce 20:5 ω3 and 22:6 ω3 from both 18:2 ω6 and 18:3 ω3.

Although *Artemia* have the ability to perform these bioconversions, these modifications probably do not represent a major change in their nutritional value as food organisms, since direct accumulation is the major means by which they obtain their component LC PUFAs and the bioconversion levels are so low. It is imperative, therefore, that culturists feed their *Artemia* a diet that contains 20:5 ω3 if they want to rectify a deficient fatty acid profile of a naupliar strain or produce larger *Artemia* that contain the same fatty acid composition as an adequate nauplii source for early life stages of the consumer organism. For example, the use of an unsupplemented rice bran diet for the mass culture of *Artemia* results in animals that contain quite low levels of 18:3 ω3 and hardly more than a trace of 20:5 ω3 (Dobbeleir et al. 1980; Sorgeloos et al. 1980). *Artemia* with this type of fatty acid profile might well be a poor diet for both freshwater and marine fish and crustaceans (Watanabe et al. 1978). Furthermore, adult *Artemia* that have been fed diets that are not fortified with 20:5 ω3 may produce cysts that have qualitative and quantitative deficiencies in fatty acids which could affect egg viability (Versichele and Sorgeloos 1980) and the value of the resulting nauplii as a food source.

The biosynthetic capability of marine fishes is generally known to be much lower than that of trout (Owen et al. 1975; Kanazawa et al. 1979; Yamada et al. 1980). There appear to be substantial differences in biosynthetic capability among various marine fishes. For example, Kayama et al. (1963a) demonstrated that the kelp bass (*Paralablax clathratus*) could elongate and desaturate 18:3 ω3 to 22:6 ω3 using mechanisms known to function in mammals. On the other hand, the red sea bream (*Chrysophus major*), the rockfish (*Sebastiscus marmoratus*) and the globefish (*Fugu rubripes rubripes*) (Kanazawa et al. 1979), the plaice (*Pleuronectes platessa*) (Owen et al. 1972), and the turbot (*Scophthalmus maximus*) (Owen et al. 1975; Cowey et al. 1976) have all been shown to have a very limited ability to modify 18:3 ω3 to 22:6 ω3. Yamada et al. (1980) found a limited ability for bioconversion in the red sea bream, black sea bream (*Mulio macrocephalus*), striped mullet (*Mugil cephalus*), and opaleye (*Girella nigricans*); however, they noted that the diet fed prior to the labeling study (a diet with a high level of LC PUFAs) may have resulted in the observed low bioconversion levels. A moderate amount of label movement, from [1-^{14}C]18:3 ω3 to 22:6 ω3, has also been shown for the ayu (*Plecoglossus altivelis*) and the eel (*Anguilla japonica*) (Kanazawa et al. 1979). In comparison with other marine fish, the winter flounder appears to have a moderate ability to bioconvert 18:3 ω3 to 22:6 ω3.

The results presented here could lend understanding to previous survival and development results obtained for various

marine organisms fed different geographic strains of *Artemia*. Some of the strains that produced poor survival contained high levels of 18:3 ω3 and little or no 20:5 ω3.

Since winter flounder fed *Artemia* that contained predominantly 18:3 ω3 as the most unsaturated fatty acid can bioconvert it to 20:5 ω3, and to a lesser degree 22:6 ω3, it appears that the lack of 20:5 ω3 in various *Artemia* strains would not be the only cause of the poor performance obtained in the flounder cultures (Klein-MacPhee et al. 1980, 1982). This tends to support the proposed theory of Schauer et al. (1980) that a fatty acid deficiency alone (e.g. a lack of 20:5 ω3) was not the single cause of the poor performance. They suggested that there may be a synergistic interaction between an organochlorine contaminant and a fatty acid deficiency that caused the poorer performance in organisms undergoing a pronounced metamorphosis. This synergistic interaction has yet to be studied; however, the data presented herewithin shows that the fatty acid profile of cultured *Artemia* can be modified in a predictable fashion, so that now these two features can perhaps be uncoupled.

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NOTES

Limitations of Using Otoliths Recovered in Scats to Estimate Prey Consumption in Seals

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Of three length classes of herring, *Clupea harengus* (30–35, 20–25, and ≤15 cm total length), fed to a captive harbor seal, *Phoca vitulina*, otoliths were recovered only from herring 30–35 cm long; of these otoliths only 4% were recovered. Using surface area to volume ratios of otoliths, other fish species were predicted to be similarly underrepresented in scats. After digestion, otoliths showed fewer annuli and a reduction in length and width. Herring otoliths in harbor seal scats indicated the consumption of large herring (≥30 cm total length) only and could not be used to estimate numbers of prey consumed. The partial digestion of herring otoliths will result in underestimates of fish age, length, and weight if calculations are based on standard relationships between these parameters and measurements from undigested otoliths.

Des trois catégories de longueur de harengs, *Clupea harengus* (longueur totale : 30–35, 20–25 et ≤15 cm), composant la nourriture d'un phoque commun, *Phoca vitulina*, captif, seuls 4 % des otolithes des poissons mesurant de 30 à 35 cm ont été récupérés. À l'aide des rapports entre la superficie et le volume des otolithes, on a prédit que d'autres espèces de poisson ne seraient pas bien représentées dans les fèces. Après la digestion, moins d'anneaux étaient présents sur les otolithes dont la longueur et la largeur étaient réduites. Leur présence dans les fèces a révélé que les phoques se nourissaient seulement de gros harengs (≥30 cm de longueur totale) et on n'a pu s'en servir pour calculer le nombre de proies consommées. La digestion partielle des otolithes entraînera des sous-estimations de l'âge, de la longueur et du poids du poisson si les calculs sont basés sur les relations normales entre ces paramètres et les mesures relevées sur des otolithes non digérés.

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The contents of scats have been used to investigate the diets of several species of pinnipeds (Carnivora): Antarctic fur seals, *Arctocephalus gazella* (North et al. 1983); Grey seals *Halichoerus grypus* (Anderson et al. 1974); leopard seals, *Hydrurga leptonyx* (Hofman et al. 1975); southern sea lions, *Otaria flavescens* (=*byronia*) (Hamilton 1934); harbor seals, *Phoca vitulina* (Everitt et al. 1981; Brown and Mate 1983); and California sea lions, *Zalophus californianus* (Everitt et al. 1981; Bailey and Ainley 1982). With the exception of leopard seals, fish comprise a significant part of the diet in all cases. Teleost sagittal otoliths in scats can be identified to the level of species, annuli on otoliths can be enumerated for aging, and fish weights and lengths can be estimated from otolith dimensions (Frost and Lowry 1981, North et al. 1983). Although it seems practical to use scats in place of more direct methods of diet determination, such as the analysis of stomach contents, some studies indicate that the digestion of otoliths will bias results (Prime 1979; North et al. 1983).

Herring (*Clupea harengus*) forms a significant component in the diet of the harbor seal in eastern Canada (Boulva and McLaren 1979). The primary objective of this study was to form a basis for the interpretation of herring otoliths in harbor seal scats. We report the recovery rate and changes of otoliths in the scats of a seal under controlled feeding conditions. Using surface area to volume ratios of otoliths, we predict relative recovery rates of otoliths from several fish species.

Methods

A 12-yr-old male harbor seal, weighing about 90 kg, was

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used as the experimental animal. The seal had been in captivity since its capture as a pup and was housed at the Huntsman Marine Laboratory in St. Andrews, New Brunswick, when the study was conducted. During the experiments the seal was kept in an open, quadrangular, dry pen whose perimeter measured 221 × 170 × 253 × 68 cm. The floor of the pen consisted of plastic-coated wire mesh (4.5 × 4.5 cm) supported on a wooden frame, thus elevating the seal about 9 cm off a concrete deck. This arrangement permitted scats to fall through the mesh. A light spray of water was used to keep the animal cool, and during 1-d intervals between experiments the seal was released to an adjacent pool.

Experiments normally lasted 3 d. Feedings consisted of 3 kg of herring per day from one of three length classes (30–35, 20–25, ≤15 cm total length). The average number of otoliths ingested per feeding was 15.0, 30.5, and 90.0 for the 30–35, 20–25, and ≤15 cm length classes, respectively. The herring fed on the first day of each experiment had one of their sagittal otoliths removed. A lateral, partial dissection of a fish's head exposed one of the sagittae with minimal disturbance to the remaining otolith and its surrounding tissues and hard parts. An examination of right and left sagittae indicated no significant difference in their lengths ($n = 30$, $P = 0.99$, paired t-test on $\log_{10}$ transformed data); therefore, otoliths were removed from either side of the head. This sample of otoliths was used as a control for the otoliths left in the fish and later passed by the seal. During the other 2 or 3 d of each experiment the seal was fed decapitated herring so that no other otoliths would be present in its scats, which were collected on the last day. For 2 d prior to the first experiment and during the 1 d between experiments, the seal received decapitated herring only. This ensured that all otoliths had been cleared from the seal's gastrointestinal tract before the start of an experiment. Forty-eight hours was deemed sufficient for the clearance of otoliths, as Prime (1979) found that the total time between ingestion and egestion of all gadid (Gadidae) otoliths by a harbor seal was 6–30 h.

Two experiments were conducted using each length class of herring. One experiment, using 30–35 cm fish, was extended to 4 d to ensure that all of the ingested otoliths were being cleared by the third day. To examine how removing one otolith from each fish may have affected the subsequent recovery of the remaining otoliths in scats, intact 30–35 cm herring were fed to the seal for 3 d. The extra set of feedings was a control for the six experimental feedings in which partially dissected herring were used.

At least 95% of each scat was recovered (based on visual estimates). Scats were stored separately and either frozen for later analysis of examined within 24 h. Otoliths were recovered by soaking scats in a solution of carboxymethylcellulose and alcohol and then washing them through nested sieves, as described by Treacy and Crawford (1981). Otoliths recovered from the scats and the control otoliths previously removed from the fish were mounted on plastic trays with a mixture of 40% Diatex (Scientific Products, Illinois) and 60% toluene. The ages of the mounted otoliths were determined by counting annuli. Otolith lengths were measured along the greatest distance of the long axis, and widths were measured along the greatest distance perpendicular to the long axis and parallel with the sagittal plane.

Results and Discussion

Only two otoliths, both from one 3-d experiment using herring from the largest (30–35 cm) length class, were recovered from scats. The recovered otoliths represented 11% ($n = 18$) of those ingested during the experiment, and 4% ($n = 54$) of the otoliths from fish 30–35 cm long ingested during the entire study. Prime (1979) also recovered a negligible proportion of herring otoliths from the scats of a harbor seal under controlled feeding conditions. However, he recovered 86% of all gadid otoliths fed to the same seal. Prime concluded that the use of scats will tend to underestimate the occurrence of small fish in pinniped diets.

The absence of otoliths from scats did not appear to be an artifact of the experimental methods. Otoliths were probably not being retained by the seal for more than 48 h. No otoliths were recovered from the experiment that lasted 4 d (allowing 72 h for otolith clearance), during which the seal was fed 30–35 cm herring. There appeared to be no decreased recovery of otoliths associated with the partial dissection done on each fish to remove an otolith. The control session, in which 30–35 cm herring were fed to the seal, intact, also produced no otoliths. Therefore, we assumed that the otoliths that were not recovered in scats within 48 h after ingestion were either totally digested or digested beyond recognition.

From the low and highly variable rate at which otoliths were recovered from scats, it is apparent that the otoliths were not reliable indicators of herring in the seal's diet. We concluded that scat examination has some utility only for indicating the occurrence of large herring (≥30 cm total length) in harbor seals' diets and that estimates of total numbers of fish ingested cannot be reliably obtained. Consequently, the analysis of harbor seal scats by Brown and Mate (1983) probably underestimated the importance of herring in the seals' diets. Pitcher (1980) reported that there was no significant difference between the number of stomachs and large intestines containing herring and other species' remains (primarily otoliths) for the 351 harbor seals that he sampled. Pitcher implicitly assumed that feces in large intestines and scats are similar with respect to the

TABLE 1. Probability of complete dissolution of otoliths of prey species of harbour seals (*Phoca vitulina*) in the Northwest Atlantic, given in decreasing rank order. The rank order was based on surface area to volume ratios, with surface area data obtained using a camera lucida (concave side of the otoliths only) and an approximation of volume obtained by weighing the otoliths with an electrobalance. Data are based on measurements from adult fish only.

Common name	Species name	Total length (cm) (n)
Mackerel	*Scomber scombrus*	37–38 (2)
Herring	*Clupea harengus*	<35 (4)
Ocean pout	*Macrozoarces americanus*	66 (1)
Herring	*Clupea harengus*	>35 (1)
Argentine	*Argentina silus*	18 (1)
Wolffish	*Anarhichas lupus*	48–57 (2)
American sand lance	*Ammodytes americanus*	26–28 (3)
Yellowtail flounder	*Limanda ferruginea*	26–40 (3)
Sea raven	*Hemitripterus americanus*	53 (1)
American plaice	*Hippoglossoides platessoides*	41–44 (3)
Silver hake	*Merluccius bilinearis*	32–47 (3)
Offshore hake	*Merluccius albidus*	41–49 (3)
Redfish	*Sebastes marinus*	30–45 (3)
Red hake	*Urophycis chuss*	29–42 (3)
Pollock	*Pollachuis virens*	63–83 (3)
Cod	*Gadus morhua*	46–56 (3)
Haddock	*Melanogrammus aeglefinus*	72–82 (2)

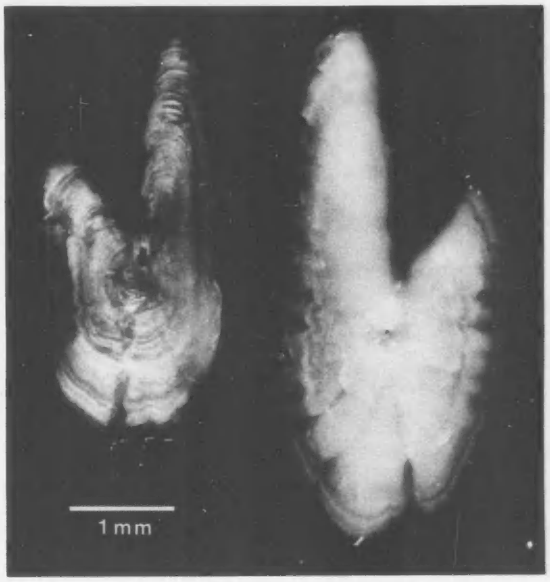

FIG. 1. Otolith recovered from a scat (left) and representative control otolith (right). Both are from herring (*Clupea harengus*) 30–35 cm total length.

undigested materials contained within them. If this is true, his results together with ours imply that herring may be equally underrepresented and misrepresented in stomachs, possibly as a result of rapid otolith digestion in the stomach.

The low recovery of herring otoliths may be due to their small size and hence high surface area to volume ratio, which subjects them to a high degree of dissolution. Table 1 shows some of the known fish prey of harbor seals in eastern Canada (Boulva and McLaren 1979) listed in order of decreasing otolith surface area to volume ratios. From this table we predict that the otoliths of mackerel (*Scomber scombrus*) and ocean pout (*Macrozoarces americanus*), like those of herring, will have very low recovery rates in scats. The low ratios of gadid otoliths may explain the high recovery rate of these otoliths reported by Prime (1979). Likewise, the intermediate ratios of the other species listed suggest intermediate rates of otolith recovery.

Both of the recovered otoliths were noticeably smaller and less opaque than their controls (Fig. 1). The increased clarity allowed most growth rings to be seen, but made it difficult to differentiate between annuli and other periodic structures (J. Hunt, Biological Station, St. Andrews, N.B., pers. comm.). As a result, the estimated ages of the recovered otoliths were less accurate than those of the controls; however, the apparent ages of the recovered otoliths were generally less than those of the control otoliths from the 30–35 cm length class (Fig. 2). This suggests that the recovered otoliths' ages were underestimated because of lost annuli. The smaller of the two otoliths showed a 1.35-mm or 26.5% reduction in length relative to a mean length of 5.09 mm calculated from the 30–35 cm fish control sample (Fig. 2). Similarly, the larger otolith showed a 0.66-mm or 12.9% reduction in length. The percent reduction in width was greater for both otoliths: the smaller otolith decreased 0.80 mm or 32.0% relative to a mean width of 2.50 mm calculated from the 30–35 cm fish control sample (Fig. 2); the larger otolith's width was reduced by 0.63 mm or 25.2%. The percent reductions in otolith length and width are consistent with the 16–47% reductions in thickness that Prime's (1979) data show for gadid otoliths. North et al. (1983), through a subjective assessment, arrived at an approximate 20% reduction in length for the otoliths of various Antarctic fish species found in the scats of Antarctic fur seals. Our results confirm the conclusion of North et al. (1983) that the partial digestion of otoliths may result in gross errors in estimates of fish weights. Likewise, estimates of fish ages and lengths may be underestimated.

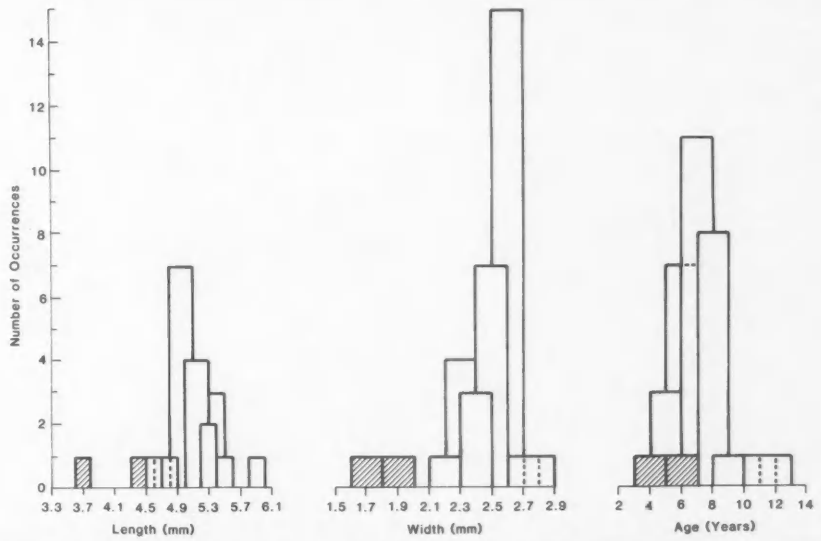

FIG. 2. Frequency distributions of various otolith characters from herring (*Clupea harengus*) 30–35 cm total length. Hatched bars represent the otoliths recovered from scats and open bars represent the control sample of otoliths. Bars were centred on the measurements that they represent.

While the results of this study are based on one animal only, they are sufficiently unambiguous to suggest that generalizations may be made to wild populations. Otoliths cannot be used reliably to reconstruct the diets of pinnipeds completely. Our results suggest that some prey species may not be represented by otoliths in scats.

Acknowledgements

We thank Susan Farquharson for assisting with the scat examinations and Peter Perley and Norman McFarlane for assisting with the construction of the seal holding area. J. J. Hunt interpreted the ages of the otoliths. We thank Drs. D. Bowen, D. M. Lavigne, W. Stobo, and J. M. Terhune for constructive criticisms of earlier versions of this manuscript. J.d.S. received support from NSERC grant A8129 to Dr. J. M. Terhune. The study was supported by funds from the Marine Fish Division, Department of Fisheries and Oceans. We thank Dr. J. Foster and the staff of the Huntsman Marine Laboratory for their cooperation. This study would not have been possible without the cooperation of Henry the seal.

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BOOK REVIEWS/COMPTES RENDUS

Evolution of Fish Species Flocks. *Edited by Anthony A. Echelle and Irv Kornfield. University of Maine at Orono Press, Orono, ME. 1984. x + 257 p., illustrations. $20.95 U.S. paperback, $28.95 U.S. cloth.*

In lakes scattered in tropical and boreal regions of the world, assemblages of fish species are found that are more closely related to one another and often more diverse than related species assemblages outside the lake. The question of the origin and evolution of these species assemblages or flocks has long tantalized biologists. This is a fresh and exciting reexamination of the evolution of species flocks by 20 investigators in 18 contributions. The book grew out of a symposium held at the 1983 American Society of Ichthyologists and Herpetologists annual meetings in Talahassee, Florida.

Topics dealt with in the volume include definition of species flock, taxonomy, evolutionary rates, genetics, ecological differentiation, sympatric versus allopatric speciation, molecular versus morphological change, allometry, and the effects of sexual selection. Species flocks in the Philippines, the Andes, Mexico, African rift lakes, Recent North American Great Lakes, and Mesozoic North American great lakes are included. Although three papers are given on Mexican and seven on African species flocks, the variety of approaches with diverse faunas makes for interesting reading.

Ribbink defines a species flock as an assemblage of closely related species endemic to a narrowly circumscribed area, which are numerous compared with surrounding regions and which apparently evolved rapidly. But Barbour and Chernoff, in discussing the "pescados blancos" (white fish) from Lake Chaplo, Mexico, agree with H. Greenwood that the central criterion for defining a species flock must be monophyly *sensu* Hennig and they consider that even a single polymorphic species can constitute a species flock.

Almost all the papers discuss species flocks in lakes although Lynne Parenti's *Orestes cuvieri* species complex did include one nonlacustrine species of fish and Dominey treated Hawaiian *Drosophila* as a species flock. Why should species flocks be more common in lakes than in rivers? According to Smith and Todd, speciation processes differ fundamentally in lakes and rivers. Speciation in lakes revolves around reproduction at different localities and times — reproductive allopatry and allochrony, and the phenotypic differences largely involve morphological traits related to food capture. They contrast this with geographic isolation in separate river headwaters where the fish are differentiating in similar habitats and under similar selective regimes; in lakes, species diverge by homing to different depths within a lake. Anthony and Echelle suggest that whereas rivers may be sufficiently heterogeneous in habitat, rivers are too unpredictable in some time frame for differentiation to take place. This latter argument has been previously used to explain the diversity of tropical versus arctic regions.

Dominey suggests that rivers are generally unstable and fluctuate seasonally, that rivers require many generalist adaptations — militating against the evolution of flocks of specialists. G. Svärdson (1979. Inst. Freshwater Res. Drottningholm Rep. 57) noted that even in Arctic whitefishes, splitting into species groups occurred in lake-spawning species; genuine anadromous or river forms are much more stable and are less widely distributed. Other questions are not asked in this volume. Would some riverine species groups of darters of the genus *Etheostoma* and of the shiner genus *Notropis* qualify as species flocks? Are there species flocks in the sea, and if not why not?

Dominey argues that lacustrine speciation may result from specific mate recognition systems which are more likely to undergo rapid divergence in allopatry than nonsexually selected traits. I wonder whether rapid sexual recognition selection is restrained by the requirement that the mate must still be able to encounter and recognize the mutant; with too wide a divergence, the sexes will not mate with one another.

Cladism evolved in the face of study of broader sets of real data. Anthony and Echelle openly consider an unresolved trichotomy as possibly an (as yet unresolved) double dichotomy, possibly of hybrid origin, or (almost heresy) real. And McClure, Thomson, and Olson found that five of eight tree nodes were polytomies rather than dichotomies — despite completeness and high quality of the specimens — and they suggest that polytomies in species swarms may represent true character distributions and not simply incomplete analyses. The question of how real polytomies can be distinguished from unresolved dichotomies is not clearly addressed. I think the only reply is a probabilistic pragmatic one. For example, as the preponderance of random samples of characters from the genome accumulates in favor of a trichotomy as opposed to a dichotomy, so it becomes increasingly probable that there was a real trichotomy and not an unresolved double dichotomy. To those who argue that the trichotomy might be reinterpreted as two dichotomies if we examined more characters, I reply that it is the same with all classification — one sample of characters might indicate that A is more closely related to C than to B, but a larger sample might suggest that A is more closely related to B than to C. The probabilistic theorem has important implications: an effort should be made to select characters from functionally unrelated characters in diverse suites such as color, morphology, behavior, biochemistry, etc.; taxonomists should table all characters for all taxa studied, including those whose polarity could not be determined, to document that characters actually used in cladograms were random and not selected.

The question of whether lacustrine species flocks evolved *in situ* in one basin (sympatrically), or when lake basins divided during lower water levels (allopatrically), or by divergence of immigrants (allopatrically) is still under debate, and different authors give different replies. However, light is shed on some cases. Intralacustrine evolution of cottids is suggested for Pliocene Lake Lahonton; all species were endemic to that lake and are missing from other nearby Pliocene lakes sampled, and only one surviving relative is known outside of the basin. McKay and Gray demonstrate significant gaps and stretches of habitat in

Lake Malawai, i.e. a 99.5-km stretch of sand and weed habitat. They found that populations of rock cichlids on either side of this sand-weed barrier did have different gene frequencies. So gaps in habitat may constitute intralacrustrine barriers to gene flow.

Data on lacustrine species flocks argue against saltatory speciation. Humphries found that 98% of genetic diversity in Mexican pupfishes occurred within rather than between samples of each species. Sage, Loiselle, Basasibwaki, and Wilson found strikingly low protein and relatively high morphological divergence in Lake Victoria cichlids (Nearctic centrarchids show more balanced diveregence in these character sets). Liem and Kaufman surprisingly found that patterns of motor inputs to the pharyngeal mill of an African and a Nearctic cichlid were strikingly similar. Straus found that the differences in relative size and shape of cephalic components represent simple scalings of shape based on size — thus, notable morphological differences may result from different sizes, or timing of allometric events and do not require large genetic changes.

The last chapter by Kornfield and Echelle, entitled "Who's tending the flock," pleads that it is the professional responsibility of all who study species flocks to be involved in ensuring the preservation of endemic faunas. They note, for example, that a major oil reserve has been found under Lake Tanganyika and that the introduction of Nile perch may be beginning to affect diversity of endemic taxa in Lake Victoria.

This book is well written and edited. It contains a number of different and current points of view of specialists in genetics, taxonomy, ecology, and morphology. It is good, exciting, and educational reading. I recommend it to specialists and students to broaden their points of view.

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Trophic Interactions Within Aquatic Ecosystems. *Edited by D. G. Meyers and J. R. Strickler. AAAS Selected Symposium 85. Westview Press Inc., Boulder, CO. 1984. 472 p. $35 U.S.*

Zooplankton may be central elements in the economies of lakes and seas; they are certainly central elements in the sciences of limnology and oceanography. Zooplankton ecologists write a substantial portion of our journals, cram the largest sessions in our meetings, and periodically summarize their progress in symposia. The best known of these is the Dartmouth Symposium of 1978 (Kerfoot 1980). The present volume, despite its ambitious title, is a sequel, focussed largely on the role of metazoan zooplankton in the trophic relations of lake communities. The next update, from the Plankton Ecology Group meeting at Plön (Lampert 1985), went to press in February 1985. The available volumes beg comparison.

The Dartmouth meeting had more contributors and was far wider in scope than the AAAS Symposium held at Toronto in January 1981 upon which this photo-offset volume is based. Essentially all of the present contributors are North Americans and most are prolific writers whose views are widely known. In fact, 40% of the present authorship also contributed to the earlier book. In consequence, while few of the contributions contain very surprising insights, most provide a cross-section of the evolving views of some of our leading workers. It is therefore regrettable that publication was delayed for four years because some then-new ideas and facts have since found alternate routes into the literature.

The book comprises 14 papers, plus an introduction by editor Meyers, a conclusion by editor Strickler, and a brief letter from G. E. Hutchinson dedicating the whole to Luigi Provasoli. In the first section entitled "Phytoplankton," the Kilhams, McCarthy and Altabet, and Lehman describe the interactions among nutrient use by phytoplankton and spatio-temporal scales of nutrient supply and turnover. Since the authors hold consistent views, this section appears either one-sided or well-integrated, depending on the reader's point of view.

The second and largest section, "Zooplankton," includes Paffenhöfer's experimental appraisal of food selectivity by calanoids and overviews of experimental studies in food selection by rotifers (Gilbert and Bodgan), and lipid storage and use by cladocerans (Goulden and Henry). The remaining two contributions in this section review the problems imposed by life at low Reynold's numbers: Koehl provides a remarkably clear analysis of particle capture and Strickler a wide-ranging discussion of how the viscosity of water might influence zooplankton predation, grooming, perception, and evolution at the interface between low and medium Reynold's numbers. These two papers represent almost 20% of the symposium. Among the interesting additions to the evolving controversy over the mechanism of suspension feeding were Koehl's explicit statement that calanoids sieve particles from small volumes using their setae and her suggestion that setular spacing may be significant in food selection; Strickler further allows that cladocerans actually filter food from the water, and both suggest that calanoids behave in qualitatively different, if undefined, ways when feeding on small particles. While there is no longer any question that zooplankton are only simple pumps-and-filters, that model seems to apply to at least some of their behaviors.

Two papers fill the "Fish" section: O'Brien and Wright compare two models describing the selection of zooplankton by their fish predators and conclude that their own model, based on separate components of predation and a consideration of relative prey size, yields a better approximation of the feeding behaviour of white crappie than does a competing model based on the reactive volume (Eggers 1982). In the second contribution to this section, Liem uses the functional morphology of fish feeding structures to challenge many standing concepts in trophic ecology including niche theory, optional foraging, and concepts associated with dietary specialization and generalism.

The four papers in the final section, "Communities," are highly varied. Meyers examines the versatility of three cladocerans (*Daphnia*, *Chydorus*, and *Alona*) in switching between littoral and pelagic resources. Kerfoot and DeMott have manipulated predator types in planktonic food webs and interpret their results to suggest that "keystone predators" may allow "vaulting," the development of "subordinate food webs" or food web "modules," by destroying the monopoly of effective competitors. Sprules and Knoechel adapt size structure analysis of plankton communities from marine workers to approach aquatic trophic relations operationally. Although average "biomass spectra" for their 37 Ontario lakes were subsequently presented in Sprules et al. (1983), their empirical relation between nannoplankton and phytoplankton biomass confirms the relation based on heterogeneous, worldwide data (Watson and Kalff 1981) at a local scale and is an interesting contribution by itself.

Finally, Hecky uses data from trophical lakes to explore some of the limitations of size structure analyses.

It must have been an interesting symposium. The contributors are authoritative and their contributions varied. Some authors chose to review largely their own work (O'Brien and Wright, Gilbert and Bogdan, Goulden and Henry); others review a larger sweep of their fields of expertise (Koehl, Strickler, Liem, Lehman, and Kilhams, McCarthy and Altabet). Those who provide original data give a concrete balance to the whole. In every symposium, one hopes to gain significant new insights into disciplines peripheral to one's own. This "dark horse" role has been amply filled by Liem's intriguing search for ecological relevance in the morphology of fish.

If a common thread can be identified in the diverse parts of the symposium, it is that each contribution describes some aspect of the search for new theories — pattern search, pattern recognition, pattern quantification, and test of this quantification. For example, the phytoplankton section illustrates an early stage in science, before even rough hypotheses are formed, when scientists set out vast research programs from which they hoped to intuit patterns and hypotheses. Others identify tentative patterns like the effects of lake physico-chemical characteristics on zooplankton size (Sprules and Knoechel) or Hecky's rules of pelagic food web structure (predators feed selectively within a wider range of smaller prey; larger species are selectively disadvantaged; higher trophic levels are more effectively exploited by man). In contrast, zooplankton ecologists typically build numerous hypotheses but these often sacrifice generality for specificity and their contributions consequently lack unity. The nannoplankton–phytoplankton relation of Sprules and Knoechel appears to be the only well-articulated, quantified theory of general applicability offered in the symposium. Once that stage is achieved, the theory should be tested; this phase of science is illustrated in Paffenhöfer's studies of filtering rate and Liem's critiques of contemporary ecological theory. A final phase of science may be the replacement of empirical theories with explanatory theories (Rigler 1982). Ecology rarely extends to this stage but ecologists compensate by offering explanations before the pattern to be explained is established. The critical reader will find this pathology in "Trophic Interactions within Aquatic Ecology," too.

Who should buy this book? It is not a review for the outsider, since the context of its various questions is rarely made explicit. There are too few contributors to provide a comprehensive overview for beginning graduate students — they should look to the Dartmouth Symposium instead. Specialists will want to read particular sections like the three papers on phytoplankton, the two on low Reynold's numbers, and those on size structure. Chapters by Hecky, the Kilhams, Koehl, Liem, and Lehman are excellent entry points to their literatures by virtue of their bibliography and no chapter is uninformative nor even uninteresting. However, few will find that this is so useful a reference book that they will need it on their own personal book shelves. Those with interests in particular fields should probably look first to interlibrary loans.

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Methods for the Study of Marine Benthos. 2nd. ed. *Edited by N. A. Holme and A. D. McIntyre. Blackwell Scientific Publications Inc., Palo Alto, CA. 1984. $44.00 U.S. ISBN 0-632-00894-6.*

Biologists interested in marine benthic communities will welcome this second edition of the book 12 years after the first edition. In the original volume the preface indicated that the aim was to bring aid and comfort to the newcomer, the isolated worker, and those in unrelated disciplines with a transient interest. As a convert to the marine realm in a relatively isolated situation, I can appreciate the wide-ranging content of the volume, which includes chapters on position fixing, photography and television, and diving as well as the usual consideration of gear and sampling design. Phytobenthos and meiofauna are also considered. With so broad a coverage, it is impossible to evaluate all the aspects of this volume, but colleagues suggest that chapters such as that on position fixing are well documented and up to date. The second edition reflects the present trend towards applied studies in impact prediction, monitoring, and evaluation as well as updating the previous information.

Some recent methodological advances (use of DNA, sediment pigments, CHN analyzers, measurement of heat production from community metabolism) are not included, but these may be regarded as advanced methods not appropriate to the original concept. However, there is reference to photographic and television methods of some sophistication, and submersibles receive a rather sketchy mention. A wide range of traditional sampling devices are described, even though some have limited use, and the section on macrofauna techniques is written with an eye to those practical hints that the beginner really needs. Gear used mostly by limnologists is largely ignored, even where it might be useful, as in coring techniques.

In terms of survey design, one might challenge the idea that species need not be identified in applied studies if "the significance of the changes at the species level cannot be evaluated." I have always had difficulty with the concept that the number of species present could be determined without actually identifying them — particularly obvious to those working with oligochaetes, perhaps, but surely a dangerous idea. Hence, the performance of ordination and cluster analysis (so much preferable to the largely redundant diversity indices) depends on the discrimination of species as the basic units of information in the data. While identifying is an expensive task, the cost should be weighed against the cost of obtaining the samples and the potential for environmental damage in the situation concerned. Surveys need not always maximize the variation due to natural gradients, as suggested. Whereas the tradition with shore transects, as in the lake surveys, is to describe the fauna along the

obvious gradient, there are situations in which sampling needs to be arranged so as to minimize variation of those parameters not related to a specific study.

The volume closes with an extensive section on energy flow measurements. This begins with an odd statement to the effect that other studies are of necessity qualitative, but as specific reference is made to growth rates, for example, it is hard to know what the statement means. There follows an exhaustive treatment of the subject, but it is noticeable that the examples are largely hypothetical, the emphasis is theoretical and less immediately practical than the foregoing. There is a final section on laboratory methods in which such practical assistance is provided, however.

This book will remain a standard reference in marine biology laboratories for many years.

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Diseases of Marine Animals. II. Introduction (O. Kinne). Bivalva to Scaphopoda (G. Lauckner). *Edited by O. Kinne. Biologische Anstalt Helgoland. 1983. DM 7500.*

The second volume of this book contains the most thorough compilation of the literature ever assembled on the diseases, both infectious and noninfectious, of bivalve molluscs. Indeed, the volume would be well worth having just for the 82 pages of literature citations on bivalves. I am amazed at Dr. Lauckner's ability to sift through the voluminous, often conflicting literature on major oyster epizootics and come up with correct interpretations, despite not being actively involved in oyster disease research.

However, I do not agree with all his interpretations and in a few cases he is clearly mistaken. For example, his statement (p. 592) that we (Pauley et al. 1967) were mistaken in concluding that *Ancistrocoma* sp. is a secondary invader rather than a primary pathogen ignores the fact that heavy infections occurred only in oysters injected with turpentine, never in the thousands of oysters examined from the natural habitat.

Other errors are also minor. There are few typographical errors, clearly a result of careful editing and proofreading. I was bothered by the consistent use of "infested" rather than "infected." I think there is general agreement among invertebrate pathologists and parasitologists that external parasites infest a host and internal parasites infect their host, even though some consider microorganisms as infecting and macroparasites as infesting. Finally, the primary focus seems to me to be on the infectious agent or cause of the disease rather than the effects of the disease on the host. That is not necessarily wrong, but I think it reflects the difference in emphasis of parasitologists and pathologists. It also reflects, even more, as Dr. Lauckner points out, that the pathological effects of most molluscan disease organisms were until recently ignored whereas taxonomy, host records, and life histories of the parasites were studied.

Despite these minor, perhaps nit-picking criticisms, I highly recommend this volume to all invertebrate pathologists, parasitologists, and molluscan biologists. The scope is complete coverage of all known molluscan diseases (and many associations I do not consider to be diseases), the treatment is exhaustive, and I found it a pleasure to read. I also congratulate Dr. Lauckner for having the courage to undertake the monumental task of writing, as sole author, this book, and the tenacity to stick with it through the completion of Volume II.

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Notice and Call for Papers
Stock Identification Workshop
Recent Innovations and State of the Art

A stock identification workshop is being organized by the Panama City Laboratory, Southwest Fisheries Center of the National Marine Fisheries Service, and will be held at the Miracle Mile Complex, Panama City Beach, Florida, November 4–6, 1985.

The objective of the workshop is to gain a better understanding of stock identification problems and evaluate approaches to their solutions.

The workshop program will consist of three sections: (1) critical review of various methods of stock identification in a plenary session; (2) contributed paper session focusing on innovative techniques and application to fishery stock problems; and (3) panel discussion and problem solving session with an invited panel. This last session will provide participants with an opportunity to discuss particular problems and critically evaluate the various approaches. Plenary session participants include Drs. Gary Winans (meristics), Fred Utter (electrophoresis), Ron Lundstrom (monoclonal antibodies), John Avise (mitochondrial DNA), and Saul Saula (statistics of multiple techniques). Review papers and abstracts of contributed papers will be published as Proceedings of the Workshop.

A block of rooms has been set aside at very favorable rates at the Miracle Mile Complex, and participants should make arrangements directly with the hotel. Identification as a NMFS Workshop participant is necessary to secure a reduced rate: Miracle Mile Complex, 9450 South Thomas Drive, Panama City Beach, FL 32407. Telephone: 1-800-874-6613, 1-800-342-8720 (Florida only).

Correspondence concerning the workshop should be sent to the workshop committee: Drs. Herb Kumpf, Allyn Johnson, Churchill Grimes, National Marine Fisheries Service, SEFC, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32407-7499. Telephone: (904) 234-6541.

Avis et demande de communications
Atelier sur l'identification des stocks
Récentes découvertes et point des connaissances

Le Panama City Laboratory (Southeast Fisheries Center, National Marine Fisheries Service) organise un atelier sur l'identification des stocks qui se tiendra du 4 au 6 novembre 1985 au Miracle Mile Complex, Panama City Beach (Floride).

Cet atelier vise l'acquisition d'une meilleure compréhension des problèmes d'identification des stocks et l'évaluation de diverses approches à leur solution.

L'atelier sera divisé en trois parties : (1) un examen critique des différentes méthodes d'identification des stocks au cours d'une séance plénière; (2) une séance de communications centrées sur les nouvelles techniques et leur application aux problèmes d'identification des stocks exploités; et (3) une discussion et une séance d'étude de problèmes avec un groupe de chercheurs invités. Cette dernière fournira aux participants une occasion de discuter de problèmes particuliers et de faire une évaluation critique des diverses approches. Les participants à la séance plénière comprennent les Drs Gary Winans (méristique), Fred Utter (électrophorèse), Ron Lundstrom (anticorps monoclonaux), John Avise (ADN mitochondrial) et Saul Saula (statistique pour techniques multiples). Les examens critiques et les résumés de communications seront publiés sous forme de compte rendu de l'atelier.

Un groupe de chambres a été réservé au Miracle Mile Complex à un prix très avantageux. Les participants doivent réserver eux-mêmes et s'identifier comme tels afin d'obtenir le tarif réduit. On droit s'adresser au Miracle Mile Complex, 9450 South Thomas Drive, Panama City Beach, FL 32407, USA, aux numéros suivants : 1-800-874-6613 et 1-800-342-8720 (Floride seulement).

Toute correspondance relative à l'atelier devra être envoyée au comité responsable : Drs Herb Kumpf, Allyn Johnson et Churchill Grimes, National Marine Fisheries Service, SEFC, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32407-7499. Téléphone : (904) 234-6541.

CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTS PUBLICATIONS

VOLUME 37, No. 12 — article by T. A. Jackson and R. E. Hecky: *Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone*.

Page 2308, left column: 2nd line should read "Metals bound in this manner could well be unavailable to organisms."

Page 2313, Fig. 8D: *x*-axis label should read "μmol org. C/g."

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Growth histories of larval redfish (*Sebastes* spp.) on an offshore Atlantic fishing bank determined by otolith increment analysis **R. W. Penney and G. T. Evans**

Benthic macroinvertebrates modify copper and zinc partitioning in freshwater–sediment microcosms **G. Krantzberg and P. M. Stokes**

Biochemical genetic stock identification of pink salmon (*Oncorhynchus gorbuscha*) in southern British Columbia and Puget Sound **T. D. Beacham, R. E. Withler, and A. P. Gould**

Micro- and macrozooplankton on the Nova Scotian Shelf in the prespring bloom period: a comparison of their potential resource utilization **M. A. Paranjape, R. J. Conover, G. C. Harding, and N. J. Prouse**

Hypolimnetic oxygen consumption in small lakes **R. R. Fulthorpe and J. E. Paloheimo**

Patterns of epipelic algal abundance with depth, trophic status, and acidity in poorly buffered New Hampshire lakes **R. J. Stevenson, R. Singer, D. A. Roberts, and C. W. Boylen**

Influence of sea temperature upon the numbers of grilse and multi-sea-winter Atlantic salmon (*Salmo salar*) caught in the vicinity of the River Dee (Aberdeenshire) **J. H. A. Martin and K. A. Mitchell**

Influence of temperature and current speed on the swimming capacity of lake whitefish (*Coregonus clupeaformis*) and cisco (*C. artedii*) **L. Bernatchez and J. J. Dodson**

Variability of density estimates and the optimization of sampling programs for stream benthos **A. Morin**

Response of biological acoustic backscattering to ships' lights **D. Sameoto, N. A. Cochrane, and A. W. Herman**

A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles **K. L. Clark and B. D. LaZerte**

Notes

Frequency of increment formation on sagittae of North Pacific albacore (*Thunnus alalunga*) **R. M. Laurs, R. Nishimoto, and J. A. Wetherall**

Organic matter in the Gulf of St. Lawrence in winter **R. Pocklington**

Book reviews/Comptes rendus

Reproduction in whales, dolphins and porpoises. Proceedings of the Conference on Cetacean Reproduction: Estimating Parameters for Stock Assessment and Management, La Jolla, California, 28 November – 7 December 1981 (edited by W. F. Perrin, R. L. Brownell, and D. P. DeMaster) **I. A. McLaren**

Atlantic fisheries and coastal communities: fisheries decision-making case histories (edited by Cynthia Lamson and Arthur J. Hanson) **S. Fraser**

Books received/Livres reçus

Announcements/Avis

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 42, No. 8, August 1985

Volume 42, nº 8, août 1985

Articles

Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water D. J. Spry and C. M. Wood **1332–1341**

Resource partitioning and niche shifts of bream (*Abramis brama*) and eel (*Anguilla anguilla*) mediated by predation of smelt (*Osmerus eperlanus*) on *Daphnia hyalina* E. H. R. R. Lammens, H. W. de Nie, J. Vijverberg, and W. L. T. van Densen **1342–1351**

Tidal exchange of anadromous rainbow smelt (*Osmerus mordax*) larvae between a shallow spawning tributary and the St. Lawrence estuary P. Ouellet and J. J. Dodson **1352–1358**

Nutrient availability for phytoplankton production in a multiple-impoundment series J. J. Elser and B. L. Kimmel **1359–1370**

A stochastic population model for managing the Atlantic menhaden (*Brevoortia tyrannus*) fishery and assessing managerial risks D. Ruppert, R. L. Reish, R. B. Deriso, and R. J. Carroll **1371–1379**

Biomass of freshwater crustacean zooplankton from length–weight regressions D. A. Culver, M. M. Boucherle, D. J. Bean, and J. W. Fletcher **1380–1390**

Space–time correlation and its effects on methods for detecting aquatic ecological change S. P. Millard, J. R. Yearsley, and D. P. Lettenmaier **1391–1400**

Historical relationships between phosphorus loading and biogenic silica accumulation in Bay of Quinte sediments C. L. Schelske, D. J. Conley, and W. F. Warwick **1401–1409**

Changes in territorial, gill-flaring, and feeding behavior in juvenile coho salmon (*Oncorhynchus kisutch*) following short-term pulses of suspended sediment L. Berg and T. G. Northcote **1410–1417**

Effects of density and microdetritus enrichment on the growth of chironomid larvae in a small pond J. B. Rasmussen **1418–1422**

Catch-per-unit-effort: a statistical model for Pacific halibut (*Hippoglossus stenolepis*) T. J. Quinn II **1423–1429**

Bioaccumulation and bioconversion of dietary labeled fatty acids in *Artemia* and winter flounder (*Pseudopleuronectes americanus*) P. S. Schauer and K. L. Simpson **1430–1438**

Notes

Limitations of using otoliths recovered in scats to estimate prey consumption in seals J. da Silva and J. D. Neilson **1439–1442**

Book reviews/Comptes rendus

Evolution of fish species flocks (edited by Anthony A. Echelle and Irv Kornfield) D. E. McAllister **1443–1444**

Trophic interactions within aquatic ecosystems (edited by D. G. Meyers and J. B. Strickler) R. H. Peters **1444–1445**

Methods for the study of marine benthos. 2nd ed. (edited by N. A. Holme and A. D. McIntyre) R. O. Brinkhurst **1445–1446**

Diseases of marine animals II. Introduction (O. Kinne). Bivalva to Scaphopoda (G. Lauckner) (edited by O. Kinne) A. K. Sparks **1446**

Announcements/Avis **1447**

Corrections to recent publications/Corrections sur récents publications **1448**

Coming next month/À paraître le mois prochain **1449**

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